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1 OCTOBER 1978

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UNITED STATES ARMY INSTITUTE OF DENTAL RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, D.C., 20012

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US ARMY INSTITUTE OF DENTAL RESEARCH WALTER REED ARMY MEDICAL CENTER Washington, D.C. 20012

ANNUAL RESEARCH PROGRESS REPORT 10ctober 1977 - 30 September 1978

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DA Project 3A161101A91C. Task 00 In-House Laboratory Independent Research

DA Project 3S161102BS06, Task 04 Research in Biomedical Sciences - Dentistry

DA Project 3S762775A825 Task 00 Oral and Maxillofacial Sciences

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USAIDR PROJECTS, TASKS, AND WORK UNITS

(Responsible Division in Parentheses)

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FOREWORD

During FY78 the USAIDR has continued to pursue initiatives directed toward improving oral health care delivery to the soldier in the field. These initiatives include methods, materials and hardware designed specifically to meet the problems anticipated in providing effective oral health care in the combat environment. In support of our development and application efforts we are also continuing studies designed to develop—the qualitative and quantitative data needed to define and pinpoint present and future obstacles to oral health care delivery.

Promising developments in the area of hardware are the possible extension of our pulse-pressure water lavage method to decontamination of the field soldier exposed to toxic materials and an x-ray intensification device which may provide a portable system of immediate x-ray diagnosis useful under field conditions.

Developmental studies of uses for biodegradable PLA-PGA copolymers or single polymers alone as hollow organ devices, medicament-carrying wound coverings, fixation devices in maxillofacial wounds and slow-drug-release systems are continuing to show positive results.

The drug DMPS which has been found to be superior to BAL and administerable under field conditions for treating mercury and arsenic toxicity has also been found useful in nickel, cadmium and copper detoxification.

Several method studies of importance to supporting the soldier in the field inlcude ways of preventing cross-contamination in the use of N_{20} anesthesia apparata, the rapid serologic identification of infectious

organisms under field conditions and means of improving casting accuracy of oral prostheses in the field.

Studies have also continued on the levels of mercury in dental personnel and facilities, noise level hazards in dental clinics and laboratories and the basic mechanisms of bone resorption. Data indicate that we are attaining significant improvement in mercury hygiene in Army dental personnel and clinics and noise is a significant hazard whose sources have been identified and are correctable. Our NIH-supported basic studies in bone resorption have produced evidence that ceramic implant materials we have been evaluating for the resolution of bone defects may inhibit bone resorption.

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23. (U) To evaluate the CO₂ surgical laser for use in the management of oral-facial combat wounds. Available data indicate that 10-12% of combat wounds and 7% of non-combat wounds requiring hospital care involved the maxillofacial region. The successful application of the CO₂ surgical laser to microsurgery in the aerodigestive system has demonstrated a number of highly desirable characteristics relative to maxillofacial surgery. These include hemostasis, no postoperative edema, minimal postoperative

TECHNICAL OBJECTIVE, 24. APPROACH. 25. PROGRESS (Furnish Individual paragraphs identified by number. Procede leaf of each with Society Classification Code.)

(U) Wound Sterilization; (U) Wound Debridement

- surgery. These include hemostasis, no postoperative edema, minimal postoperative scarring, sterilization of infected wounds, minimal pain and a sharply demarcated operative field. This suggests that the CO₂ laser may provide a rapid, safe and superiod approach to the debridement and subsequent reconstruction of maxillofacial wounds. A more effective modality of managing maxillofacial wounds would result in significant savings in hospital costs and professional man-hours and effect a rapid
- 24. (U) A CO₂ surgical laser will be utilized to establish baseline reactions in normal oral tissues of experimental animals. Subsequently simulated maxillofacial wounds (to include teeth, bone and soft tissue) will be treated to evaluate the feasibility of using the surgical laser for hemostasis, incision, excision and debridement. The rapid sterilization of selected instruments and appliances will also be studied.
- 25. (U) (77 10-78 10) Sufficient data was collected to establish the feasibility of using the CO₂ surgical laser in the treatment of oral wounds and the sterilization of dental instruments. These studies have been transferred to our 825 program and the results are described under accession number OF 6040.

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(U) Combat Wounds; (U) Bacterial Identification;

(U) Liquid Chromatography; (U) Lipids (U) Fatty Acid Profile

21. TECHNICAL OBJECTIVE. 24. APPROACH, 25. PROGRESS (Pumish Individual paragraphs Identified by masher. Procedu lost of each with Society Classification Code.)

- 23. (U) To develop an improved, rapid, sensitive and precise method for the identification of Pathogenic bacteria associated with combat wounds. A critical phase in the successful treatment of combat wounds is the early detection and identification of potentially destructive pathogenic organisms and the institution of appropriate therapy Instrumental techniques are now available which may make it possible to identify unique constituents from different microbial genera thus offering the possibility of rapid and accurate identification of pathogenic organisms directly from body fluids and wounds. Such a procedure could eliminate lengthy and sometimes difficult culturing procedures, reduce man-hours expended in the laboratory, reduce professional man-hours in patient treatment, speed wound healing, lower morbidity and be a positive moral factor for the combat soldier.
- 24. (U) Currently used gas chromatographic methods as well as highly sensitive liquid chromatography methodology recently developed at USAIDR, will be used to identify cellular fatty acids and metabolic by-products by wound infecting microorganism.
- 25. (U) (77 10-78 10) Studies continue to show that HPLC is a superior method of identifying bacteria by this fatty acid profile. Whether or not bacterial cells maintain their unique fatty acid profile in mixed cultures is the subject of current work. Initial data indicate that there are no qualitative changes but there are some quantitative differences which may affect identification of cells in mixed cultures.

A Rapid Method for the Identification of Pathogenic Organisms Associated with Combat Wounds

Individual cellular components of microorganisms (protiens & lipids) have been found to be valuable in the identification of bacteria. Studies have been initiated to develop an improved and rapid method for the identification of contaminating pathogenic organisms associated with combat wounds and possible biological weapons. Currently used gas chromatography (GC) as well as High Pressure Liquid Chromatography (HPLC) have been utilized in the detection of specific cellular fatty acids of wound infecting microorganisms. Studies in our laboratory have shown that HPLC provides a superior fingerprint methodology in the study of bacterial fatty acids. HPLC and GLC have been applied to the study of normal and pathogenic organisms, and in pure culture it was found that these organisms could be readily identified. In our studies, new previously unidentified fatty acids have been found for one of the pathogenic organisms. In addition, of cyclopropane fatty acids does not occur in the manner as originally suggested.

One of the key questions in this study is whether the cellular fatty acids of bacterial cells maintain their unique character in mixed cultures. Our investigations have shown that they do maintain their unique character qualitatively but that in some cases they do have an influence upon each other. This indicates that their utilization in rapid identification of pathogens in wounds shows high promise and may become a valuable and very rapid tool in the combat zone.

PUBLICATIONS

Bussell, Norman E., Miller, R.A., Setterstrom, J., Gross, A.: "A Preliminary Report on the Use of HPLC in the Analysis of Bacterial Fatty Acid Composition." 174th National Meeting A.C.S. 1977 (Abstract).

Miller, Robert A., Bussell, Norman E., Setterstrom, J., and Gross, A.: "Quantitation of Long Chain Fatty Acids as the Methoxyphenacyl Esters Using HPLC." 174th National Meeting A.C.S. 1977 (Abstract).

Bussell, N.E., Miller, R.A., Setterstrom, J.A., Gross, A., Ricketts, C.: "Profiles of Fatty Acids in Mixed Cultures of Oral Streptococci."
J. Dent. Research <u>57</u>:A646, 1978, (Abstract).

Bussell, N.E. and Miller, R.A.: "Analysis of Hydroxyl Fatty Acids by High Pressure Liquid Chromatography." 176th National Meeting A.C.S., 1978.

Miller, R.A. and Bussell, N.E.: "High Pressure Liquid Chromatography of Unsaturated and Cyclopropane Fatty Acids." 176th National Meeting A.C.S., 1978.

Miller, Robert A., Bussell, N.E. and Ricketts, Charles: "Quantitation of Long Chain Fatty Acids as Methoxyphenacyl Esters." J. Liquid Chrom. 1:291-304, 1978.

Bussell, Norman E., Miller, Robert A., Setterstrom, Jean A., and Gross, Arthur: "High Pressure Liquid Chromatography in the Analysis of Fatty Acids Composition of Oral Streptococci and its Comparison to Gas Chromatography." Proceedings Liquid Chromatography Symposium 1:Biological/Biomedical Applications of Liquid Chromatography, Boston Mass. 1977 (IN PRESS).

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- (U) Salivary Protein; (U) Salivary Electrolytes

 23 TECHNICAL OBJECTIVE.* 24 APPROACH, 25 PROGRESS (Furnish individual paragraphs identified by number. Procede lost of each with Society Classification Code.)
- 23. (U) To determine if saliva can be used as a diagnostic tool in evaluating the exposure of combat troops to lethal agents. To determine if parameters in saliva can be used to monitor the progress of therapy for lethal agent exposure. Develop a rapid simplified field technique for identification of lethal agent exposure in the combat soldier.
- 24. (U) Changes in Saliva produced by lethal agent exposure will be evaluated. The particular areas of study will be protein, electrolyte and immunological components. Possible methodology developed will be evaluated in the field and at the hospital level.
- 25. (U) (77 10-78 10) Cynomologus monkeys were subjected to cholinergic drugs including GB a moderately toxic choline esterase inhibitor. Salivary composition was studied and compared with clinical signs and symptoms. Evidence was obtained that GB interfered with immunological mechanisms. These studies are a first step toward attempts to provide a rapid, noninvasive, field delivered test for nerve gas exposure.

A Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents

Five cynomolgus monkeys were subjected to various cholinergic drugs, e.g. pilocarpine and neostigmine. The animals were also subjected to atropine and TAB as antidotes to the cholinergic drugs. Finally, the animals were exposed to GB, a moderately toxic acetyl choline esterase inhibitor which could be used against United States military personnel.

Pilocarpine produced the same effects as did the neostigmine on salivary composition, although they exert their parasympathomimetic effects in a different manner, they are both cholinergic drugs.

Comparative isoelectric focusing studies on different animals to establish baseline data revealed that no animal used previously for oral model systems, (cynomolgus monkey, rhesus monkey, rat or dog) produced the same protein patterns as does man. However, the Maccaus monkey does provide an electrolyte and trace metal response similar to that of man.

The clinical signs produced by the lethal agent GB (a moderately toxic organophosphate) were entirely different from those produced by neostigmine which is a therapeutic acetyl choline esterase inhibitor. This would indicate that the effects of GB are CNS in nature and only minimally parasympathomimetic.

Experimental evidence was also found which indicated that GB interferes with the normal immunological defense mechanisms of an exposed animal. The importance of this finding in contaminated wounds is being followed.

Further studies are planned to repeat the first five animals to confirm the original findings. These important findings provide the first step in ongoing studies with several animal species, which are targeted at providing a rapid, noninvasive, field-delivered test for nerve gas exposure.

PUBLICATIONS

Bussell, N.E., Miller, R.A., and Setterstrom, J.A.: Isoelectric Focusing of Whole Saliva From Cynomolgus Monkeys. Abstract submitted, IADR March 1979.

Miller, R.A., and Bussell, N.E.: Elemental Analysis of Whole Saliva From Cynomolgus Monkeys. Abstract submitted, IADR March 1979.

Setterstrom, J.A., Bussell, N.E., Stanko, R.S., and Gross, A.: A Comparative Study of Animals and Human Salivary Proteins by Isoelectric Focusing. Abstract submitted, IADR March 1979.

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(U) Water Purification; (U) Field Dental Units;

Dayoub, M.B., LTC, DC

- (U) Water Contamination

 23 TECHNICAL OBJECTIVE. 24 APPROACH. 25 Individual paragraphs Identified by number. Proceeds test of each with Society Closelitication Contamination.
- 23. (U) To modify the Final Treatment and Operating Unit with a simple filtration system which will reservits internal water circulating system free of known microbiological warfare agents and radiobiological debris with particle size greater than 0.2m.
- 24. (U) Various primary and secondary filters of various pore sizes will be inserted into the waterline connecting the unit water reservoir and unit console. Varying filter configurations will be tested for their ability to remove microbiologic and radiologic contaminants.
- 25. (U) $(77\ 10-78\ 10)$ It has been shown that water lines of the field dental operating and treatment unit (ENCORE) can be sterilized with 2% gluteraldehyde and the sterility maintained with a combined Balston capsule filter and a secondary Swinnex 25 filter for periods up to 72 hours when using the high-speed handpiece and water syringe.

veilable to contractors upon originator's approval

Foreign Intelligence Considered

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A Rapid Water Purification System for Field Dental Units

The field dental operating and treatment unit (ENCORE) has been designed for use by the military for the delivery of dental health care in the combat zone. These units will have to be utilized in some instances in a hostile environment consisting of enemy fire, chemical and biological agents, and possibly radioactive debris. Investigations in our laboratories are underway to test the necessary modifications for successful deployment of the unit in such a contaminated environment. Previous research by the USAIDR has shown that all such water containing units become heavily contaminated during normal usage. USAIDR researchers have shown that the field dental unit is capable of being decontaminated of bacteria by two methods, the use of 2% gluteraldehyde (Cidex) and with the use of in line filters. Once decontaminated, the unit will remain free of microbial contamination of environmental origin for at least 2 weeks use. With indigenous tap water and a standard contaminant (Pseudomonas aeruginosa) in the water reservoir, we have been able to keep the high speed handpiece and water syringe of the unit free of cultivable bacteria for up to 72 hours using a Balston capsule filter combined with a secondary Millipore "Swinnex 25" filter. While the decontamination has been successful in terms of decontamination, the filters in the waterline have imposed restrictions in the flow of water. After 4 minutes of continuous use, the water flow decreased from 190 ml/minute to 60 ml/minute. No progress has been made on the removal of radiological debris from the field dental unit. Research continues.

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 23 TECHNICAL OBJECTIVE.® 24 APPROACH, 25 PROGRESS (Pumloh Individual persylvapho Identified by number Procede test of each of the Society Classificat
- 23. (U) To develop simple and fail-safe techniques for measurement of the <u>in-vitro</u> corrosion rates of newly developed dental and implant alloys. To identify through future work, the most suitable metallic substance for application in military dental and medical practice.
- 24. (U) Potentiodynamic polarization characteristics of three low-gold alloys and three base metal alloys will be determined and compared with those of a conventional Type II gold casting alloy.
- 25. (U) (77 10-78 10) A commercially available system for studying corrosion phenomenon was applied to 3 low-gold alloys and a type III gold alloy as a comparative standard. The data indicated that the 3 low-gold alloys, when placed in a model biological system exhibited corrosion properties which make them unacceptable for Army use. The in-vitro system developed appears to be an excellent approach to predicting the in-vivo performance of low-gold alloys.

Foreign Intelligence Considered

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Development of Techniques for Determination of the Corusion Potential of Dental and Biomedical Alloys

The continuing need to minimize expenditures in a member consistent with good oral health care and combat readiness has led to the study of a number of base metal and "low" gold alloys as replacements for the more expensive alloys now in use. Problems of alloy corrosion increase as precious metal content decreases. Since alloy corrosion problems are electrochemical in nature an in-vitro corrosion measurement should provide definitive data which might correlate closely to data derrived from more costly clinical evaluation and thus provide an efficient screening of potentially useful alloys. A commercially available system for studying corrosion phenomena has been applied to equally sized cylindrical specimens of test alloys immersed in Ringers solution. This model biological system was first applied to type III gold alloy as a comparative standard. Primary passive potential, critical anodic current density, region of active corrosion, onset of passivation, passivation region and transpassive region were determined for the comparison standard cious high-fusing alloy and 3 low gold alloys. The resulting data 11 les that the low gold alloys have corrosion properties which make them unacceptable for military use. A comparison of the results of the in-vitro method of evaluation with clinical corrosion data will determine the ultimate validity of the in-vitro approach.

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- (U) Air-borne Carcinogens (U) Inorganic Nickel; (U) Particulate Pollutants.
- 23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede test of each with Security Closeification Code.)
- 23. (U) To establish within USAIDR the capability to monitor and to measure the concentration of nickel and other particulate pollutants in Army Dental Laboratories.
- 24. (U) Airborne particulate matter will be collected from a metal-finishing room for a period of 35 days. Dust acquired from 5 intramural locations will be analyzed quantitatively for nickel content.
- 25. (U) (10) Initial monitoring results for a large Army dental laboratory indicates entrations of nickel between 4 and 8 micrograms per cubic meter. This is wi povernment standards for occupational exposure to nickel. Work continues.

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Evaluation of Microwave Ovens as a potential method of Sterilization

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• US Army Institute of Dental Research

US Army Institute of Dental Research
Division of Preventive Dentistry

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
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ADDRESS: Washington, D.C. 20012

NAME: LYON, T.C., COL, DC YELEPHONE: 301-677-7451

SOCIAL SECURITY ACCOUNT NUMBER:

TELEPHONE: 202-576-3484 SOCIAL ASSOCIAT ASSOCIAT

HAME: Brunner, D.G., LTC, DC

Foreign Intelligence Considered

(I KEYWORDS (Fracedo EACH with Society Classification Code)

(U) Microwave 0:

(U) Microwave Ovens, (U) Sterilization, (U) Nonmetal

Sterilization (1) Wound Dressing Sterilization
3 TECHNICAL OBJECTIVE. 24 APPROACH, 25. PROGRESS (Pumish Individual paragraphs Identified by mus

23. (U) To determine the ability of microwave ovens to destroy bacterial spores on non-metallic instruments and wound dressings and to determine if such destruction is a function of heat alone.

- 24. (U) A commercially available microwave oven with certain characterists will be tested for its ability to destroy bacterial spores as a function of time, rature, thermocouple placement and power output. A variety of materials will be for their thermal capabilities during test sterilization procedures.
- 25. (U) (77 10-78 10) Due to delay in the manufacturers delivery date this project has just begun. The particular method of achieving heat increase by microwaves, i.e. molecular motion of moisture, requires that instruments be placed in a liquid. Two liquids currently being evaluated are Alcide F an as yet unevaluated sporocide, and one of the commercial phenolic disinfectants, (Vesphene). The potential for sporocidal action of phenolics with heat is well recognized. A limitation of the microwave oven is the maximum temperature of 200°F. Work is in progress.

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(U) Gingival Healing (U) Cyclic GMP (U) Biodegradable Polymers (U) Biodegradable
13 TECHNICAL OBJECTIVE. 24 APPROACH, 25 PROGRESS (Furnish Individual paragraphs Identified by number Proceeds text of each with Society Cleanification Code.) Ceramics 23. (U) Studies conducted by the Army in recent years show that 10-12% of combat wounds involved the maxillofacial apparatus. Further, 7% of noncombat injuries requiring hospital care involve the maxillofacial region. This results in the loss of approximately 1,000,000 man-hours per year. The research objective is to accelerate or otherwise improve healing of the combat maxillofacial wound, demonstrate cost effective measures by decreasing medical requirements and hospital stay and effect a rapid return of the soldier to duty.

PRINCIPAL INVESTIGATOR (Puminh SEAN II U. MAME: Battistone, G.C., TELEPHONE: 202-576-2987

SOCIAL SECURITY ACCOUNT NUMBER:

MAME: Grower, M.F., LTC, DC

- 24. (U) Studies on the effects of biochemical and physical factors to include chelate complexes, cyclic AMP, prostaglandins, scar inhibiting agents, in vivo growth factors and electric currents on the rate of healing in soft tissue and bone will be done. The mechanism of any beneficial alteration in healing effected will be investigated and pursued to human usage.
- 25. (U) (77 10-78 10) Polylactic acid films containing cyclic AMP were found to stimulate early repair of gingival wounds. Epinephrine-containing anesthetic was found to increase gingival cAMP immediately after injection. No effect was noted in bone. Positive results suggest a positive effect on early wound healing. Studies of various combinations of biodegradable polymers and ceramics in filling bone defects indicate the formation of more dense connective tissue compared to controls. The fabrication of various wound dressings from degradable polymers is continuing. Studies on the effects of electric currents on bone healing have been terminated.

verlable to contractors upon originator's approval

NAME Cutright, D.E., COL, DC TELEPHONE: 202-576-3484

Foreign Intelligence Considered

Acceleration of Wound Healing The Effect of cAMP and PLA on Wound Healing

The effects that a dressing of PLA and PLA containing cAMP have on wound healing was studied in 8 rhesus monkeys. The model system used to study oral wound healing was a gingivectomy done on the facial aspect of the maxillary and mandibular arches from the lateral incisors to the 2nd molars.

The gingivectomies done on each animal were done in equal quadrants. Two control areas were dressed with coe pac, one experimental was dressed with PLA alone and one dressed with PLA - cAMP. The plain PLA dressing was derived from a lyophylized methylene chloride solution of PLA. The PLA cAMP combination was similarly prepared from a mixture of a methylene chloride - PLA solution with an absolute ethanol solution of cAMP. Tissue samples for biochemical analysis and histological evaluation were taken at zero time, 7 days and 14 days. No statistical differences in the cAMP and cGMP content nor of the cAMP binding activity of the repair tissue was noted when results were compared at 7 and 14 days after surgery. No effects were found on phosphodiesterase activity. Histologic analysis of the repair areas showed similar healing responses without any undue toxic reactions to any of the agents used. Statistical analysis of the changes in collagen content of the repair tissue using Friedman's 2 way analysis of variance showed that the dressing did have a statistical effect on the collagen content. It was found that the wounds covered with the dressing containing PLA and cAMP had relatively greater collagen content than that of the other dressings at 7 days. By 14 days post surgery all the repair sites showed

similar collagen content. It appears that cAMP in the wound dressing may stimulate early repair of the wounded area and thus further study is being done on the most effective means of delivery and concentration to be used to achieve this effect in combat type wounds.

Effects of Epinephrine on Bone and Gingival cGMP and cAMP Levels.

The nudeotides, cAMP and cGMP appear to be effectors of hormone action and play a role in the modulation of tissue inflammation and wound repair. In some tissues these effects have been proposed to occur due to reciprocal changes in the levels of cAMP and cGMP. This study determined the basal levels in the gingiva and alveolar bone of Rhesus monkeys and the effect that infiltration of Lidocaine containing 1:100,000 epinephrine had on them. Baseline values of cGMP and cAMP were determined by removing the facial attached gingiva from the right maxilla and mandible and 1-2 mm of underlying alveolar bone from six monkeys. The attached gingiva and alveolar mucosa on corresponding areas of the left side of the maxilla and madible were then infiltrated with 0.9 cc of Lidocaine containing epinephrine. The maxillary gingiva and bone were collected 5 min. after injection and the mandibular tissues were collected 30 min. after injection. Radioimmunoassay of the nucleotides in the excised tissues showed that the gingiva had a cGMP content of 0.39+0.05 picomoles cGMP/mg protein and a cAMP content of 20.54+4.74 picomoles cAMP/mg protein (P<0.005). The alveolar bone had 1.74+0.48 picomoles cGMP protein and 6.34+1.19 picomoles cAMP/mg protein

(P<0.005). The gingiva infiltrated with epinephrine showed an 80% increase in cAMP levels after 5 min. but no change in cGMP was noted; nor were the cAMP and cGMP levels in the alveolar bone changed. Thirty minutes after infiltration of the tissues the cAMP levels of the gingiva had almost reached basal levels and no additional changes were noted in gingiva or bone cAMP and cGMP levels. It appears that only the levels of cAMP in gingiva are affected by infiltration with epinephrine containing anesthesia and that cGMP levels do not show any reciprocal changes nor does infiltration of the attached gingiva and alveolar mucosa affect the cAMP or cGMP levels of underlying bone. Since cAMP appears to stimulate wound repair, modalities having a positive effect on cAMP levels may accelerate the repair of combat wounds.

Biodegradable Polymers and Ceramics Used As Agents to Fill Bone Defects

The healing of calvaria defects in rats filled with biodegradable ceramic (tricalcium phosphate), biodegradable polymer (50% PLA, 50% PGA) and a combination of the ceramic and polymer together as well as spherical hydroxyapatite was studied for 1, 2, 4, and 6 months. All of the agents were histocompatible and showed minimal inflammatory activity except for the spherical hydroxyapatite, which showed somewhat more response than the other agents. The wounds filled with biodegradable polymer and polymer with ceramic appeared to heal with more dense connective tissue than the control wounds. While some of the agents tested showed evidence of calcification and osseous regeneration, this was not a consistent finding in all samples. Further studies are in progress

targeted at pinpointing those agents stimulating early bone formation.

The Effect of Electric Current on Bone Healing

Data collected to date in our laboratory leads to the conclusion that any acceleration of bone healing obtained in experimental animals, using an invasive approach (placement of an electrode in the traumatized area), is highly variable, in some cases negative and usually not of sufficient magnitude to warrant the invasive approach. A recent conference on the subject, attended by the principal investigator, in general reinforced the above conclusion. Some investigators stated that their results are never negative but are indeed variable and not sufficiently remarkable in accelerating normal healing to support the invasive approach with its attendant risks. Electric currents are being used quite successfully by several groups for resolving long term non-unions in human bones. However the methodology remains highly empirical and considerable basic research in bioelectric phenomena is needed to ultimately determine if electric currents will have practical application in accelerating the normal healing process. Until such time as more fundamental data are available upon which to base application studies this task is terminated.

Fabrication of Wound Dressing from Biodegradable Polymers

Films of biodegradable polymer (polylactic acid) were formed by spraying the polymer on glass. These films were then used to cover surgical wounds in rats. In addition, polymer films were sprayed directly on the wounds in rats. Both films showed biologic compatibility and

both allowed tissue ingrowth through the films while providing initial wound protection. The methylene chloride solvent caused only minimal tissue reaction. Work continues with this promising and new method of combat wound protection.

PUBLICATIONS

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Grower, M.F. and Stow, J.A.: "Effects of Epinephrine on Bone and Gingival cGMP and cAMP Levels. Abstract submitted to IADR 1979.

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Foreign Intelligence Considered I MANE: Russell, E.A., COL, DC. Bussell, N.MAJ, DC (U) IV Valium (U) Biodegradable Polymers, (U) Antibiotic (U) Mercury (U) Sodium 2,3 Dimercaptopropane Sulfonate (U) Nitrous Oxide (U) Eugenol

23. TECHNICAL OBJECTIVE.* 24. APPROACH, 25. PROGRESS (Fuminh individual paragraphs identified by number. Procedu test of each with security Classification Code.)

- 23. (U) To evaluate the special military problems of drug storage, heat susceptibility, long-term drug potency, sterility of bulk items, lack of refrigeration in combat zones and delivery to the patient. To investigate drug hazards. To investigate the use of biodegradable polymers for the long term, slow release delivery of drugs.
- 24. (U) Experiments will be conducted to evaluate pain killing medicaments used in military dental practice. The hazards involved in the use of various drugs will be studied and improved means of drug delivery will be investigated.
- 25. (U) (77 10-78 10) Biodegradable polylactic acid in the form of rigid foams have been prepared with tetracycline, sulfanilimide and biodegradable ceramic. These preparations are being evaluated as wound dressings and space obliterators. The safe administration of IV Valium has been studied. Highly purified eugenol does not appear to differ from stock eugenol in cavity and root canal preparations. The most recent data on mercury levels in dental personnel and facilities indicate improved mercury hygiene. The drug DMPS has been found effective for nickel, cadmium and copper detoxification. It has been found safe to return patients directly to duty after nitrous oxide sedation when it is followed by a 3 to 5 min. oxygenation period.

The Problems Involved in Military Oral Health Care Delivery Related to Therapeutic Agents and Materials

Incorporation of Antibiotics and Other Agents Into Biodegradable Polymers

Biodegradable polylactic acid has been dissolved in methylene chloride and then precipitated by mixing the solution with absolute methanol. A rubbery polymer is formed which allows incorporation of powdered antibiotics or other particulate material into it. Polymers containing tetracycline, sulfanilamide, and biodegradable ceramic have been prepared for evaluation. The polymer can be shaped by putting it in molds, and after lyophilization it has a rigid foamy consistency. These antibiotic and drug containing spongy forms will be used as wound dressings and space obliterators.

The Behavior of Valium in IV Solutions

A study of the solubility of Valium in commonly used intravenous solutions showed Valium to be equally insoluble in 5% dextrose in normal saline, 5% dextrose in water, normal saline, and Ringer's lactate. However, the precipitate which was formed became completely resuspended when mixed with as little as 39 - 42% plasma in vitro. This would indicate that the chalky precipitate seen in the IV tubing when Valium is injected into a running IV near the venipuncture site becomes resuspended when mixed with plasma in vivo. If one elects to inject Valium into the tubing of a running IV, it is recommended that the drug be administered slowly to assure adequate mixing with blood plasma in order to prevent the circulation of particulate matter. This alleviates one of the major problems of

Preparation, Purification, and Use of Eugenol for Zinc Oxide/Eugenol Cements

The purity of several brands of eugenol was compared by using High Performance Liquid Chromatography with a UV detector. Greater than 95% of the impurities seen in the U.S.P. Eugenols were removed by preparative liquid chromatography. NMR sepctroscopy suggests that there may be a difference in chemical reactivity between purified and stock eugenol. Preliminary studies in cavity and root canal preparations have demonstrated no difference in the irritability of the purified and stock eugenol.

Mercury Levels in Army Dental Personnel and Facilities

A total of 176 blood and urine determinations have been done to date on army dental personnel. The most recent data obtained suggests that there has been an improvement in mercury hygiene in army dental clinics.

Data obtained in the past have indicated a positive correlation between the mercury levels found and the volume of restorative work done in a given clinic. Determinations of atmospheric mercury and mercury spillage have also indicated that less atmospheric mercury was present in clinics originally designed as dental clinics as compared to older buildings converted to dental clinics even though local concentrations around amalgamators, work tables etc. did not appear to differ significantly between clinics.

The most recent atmospheric and spillage data shows low levels of mercury in the general atmosphere of the clinics tested which do not differ significantly from past data. However a striking reduction was found in local concentrations due to spillage in and around amalgamators. Part of this reduction appears due to a new type of amalgamator which appears to be less prone to contamination. However it was also noted that the older amalgamators were in general less contaminated. A review of all the data obtained to date indicates that the contamination level of amalgamators in individual operatories is the most meaningful single piece of data obtained in predicting the mercury body burden of dental personnel. In every case examined thus far, above normal blood and urine mercury in a given individual correlated with high amalgamator contamination in the operatory or operatories used by that individual. Thus in the most recent clinics studied amalgamator contaminations were significantly and consistently lower than those found in previously examined clinics and the dental personnel in the recently examined clinics displayed consistently low blood and urine mercury values well below the upper limits of normal values.

The improvement noted in mrecury hygiene in the most recent project may be due to the greater awareness of dental personnel of the hazards of mercury. It was noted that military dental personnel displayed more interest in mercury hygiene than we have found in the past. This was especially true where testing had been done. This data has been dissemenated to the field.

Use of Na 2,3 Dimercaptopropane Sulfonate (DMPS) in The Detoxification of Nickel, Cadmium and Copper

Studies on the use of DMPS in the treatment of metal poisoning have continued. We have reported that DMPS is particularly effective in the detoxification of mercury and arsenic and is far superior to BAL, its analogue. Because of its low toxicity, crystalline form, good shelf life and effectiveness via the oral route DMPS has potential usefulness in the field soldier under minimum supervision.

The ability of DMPS to detoxify nickel, cadmium and copper in the presence or absence of mercury was determined in experimental rats.

Separate groups of adult male white rats were given IP injections of the chlorides of either nickel cadmium or copper alone or each in combination with mercuric chloride. On the day following metal injection each group was given aqueous IP injections of 30 mg/kg day DMPS for 3 days. Urine and feces were collected and analyzed daily for the appropriate metal(s). DMPS treatment resulted in mean total excretions of 10.3, 8.6 and 14.8 percent of the injected doses of Ni, Cd, and Cu respectively, an increase of five to tenfold above control levels. When given with mercury the excretion levels of Ni, Cd and Cu decreased slightly. Mean total mercury excretion was 54% of the dose injected. It is concluded that DMPS is a useful and low toxicity agent for detoxifying contaminated personnel and it may prove useful for multielement detoxification under field conditions.

Recovery from Nitrous Oxide-Oxygen Psychosedation as Determined by the Fusion Frequency of Flicker

This study was undertaken to determine if a 3-5 minute oxygenation period following administration of nitrous oxide-oxygen sedation for dental treatment would be adequate to completely reverse the psychomotor impairment seen with this modality of sedation. The results supported other studies which revealed complete recovery following a 3-5 minute post treatment oxygenation period. These findings indicate the patient can return directly to duty therefore preventing lost duty time. This information has been published and sent to the field.

Preparation and Characterization of PLA-PGA Medicament Combinations for Use in Controlled Release of Biologically Active Agents

PLA-PGA pellets containing radioactive tetracycline were implanted subcutaneously in rabbits with blood samples being taken at timed intervals over a 7 day period. Due to technical problems, the results were inconclusive. Plans are near completion for repeating the investigation, and to use different animals for the initial phase of the study.

Potential uses for these controlled-release-biological-agent pellets is unlimited in military personnel; this is especially true in the current short war scenarios. Using implantation techniques of injectable microcapsules, a variety of vaccines and/or antibiotics specifically tailored to the projected area of committment can be administered prophylactically prior to deployment to any area of potential conflict.

PUBLICATIONS

- Hawkins, C.C., Russell, E.A. and Ayer, W.A.: Recovery From Nitrous Ande Oxygen Sedation as Determined by the Fusion Frequency of Flicker. Anesth. Prog. 25(4): 121, 1978.
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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY					OE 6037	78 10 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
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RESPONSIBLE INDIVIDUAL NAME: Cutright, D.E., COL, DC TELEPHONE: 202-576-3484 21. GENERAL USE				PRINCIPAL INVESTIGATOR (Purmion SEAN II U.S. Academic Inclination) NAME: Huget, E.F., COL, DC TELEPHONE: 202-576-3092 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS				
	telligence Co	NAME: Fehrman, S., 1LT, MSC NAME: De Simon, L. Pesthesia (II) Adjunctive Drugs (II) Endorphi						

[PII Redacted]

(U) Current density Hazard.

23. (U) To develop effective electroanalgesia equipment and techniques for easier and safer management of "anesthetic risk" patients requiring immediate and extensive treatment of combat injuries of the oral-facial region. The simplicity of technique and equipment make this method ideally suited for use in field medical surgical practice, in the treatment of large numbers of patients in mass casualty situations and in the fixed dental treatment facility. The success of this project will establish cost effective measures which will eventuate in vast dollar savings.

- 24. (U) To construct equipment for the synthesis and delivery of electroanalgesic currents. This will be followed by studies designed to determine optimum currents and frequencies. Methods of application and administration through time and intensity changing currents and chemical adjuncts will be studied. The final study will involve a demonstration of the safety of the use of this equipment.
- 25. (U) (77 10-78 10) Work done to date indicates that electroanesthesia without adjunctive drugs is not effective and the current densities experienced at the site of electrode attachment are not without possible hazard. Since the neuro-chemical mechanisms involved are complex and not yet defined, additional work will be limited to an attempt to identify and correlate the resently described endorphins with observed electroanesthetic effects in the hope that some rationale beyond the purely empirical approach used at present may be evidenced.

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RESPONSIBLE INDIVIDUAL			NAME: Gross, A. COL, DC							
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- (U) Oral Streptococci (U) Dental Unit Sterilization

 (U) Anesthesia in Field (U) Bacterial Identification (U) Oral wounds (U) Phagocytic

 23. TECHNICAL OBJECTIVE. 24. APPROACH, 25. PROGRESS (Pumilah Individual paragraphs Identified by number. Proceeds lead of each with Society Classification Code.) Dysfunction
- 23. (U) To investigate the source and treatment of oro-facial infections encountered in field conditions, foreign countries and diverse climates. To evaluate the special agents, instruments and chemicals necessary under military conditions.
- 24. (U) Oro-facial infections of significance in the diverse military environment will be studied by microbiological, immunological and electronmicroscopy methods. Possible sources of oral infections will be evaluated and the effectiveness of commercially available as well as in-house designs will be studied for their ability to control or prevent oral infections.
- 25. (U) (77 10-78 10) A low-cost simplified micromethod for the identification of oral streptococci has been successfully extended to 65 strains. The microbial contamination of anesthetic machines used in the field has been studied and recommendations made for reducing patient contamination. A method of sterilizing the water lines of dental units for periods of 10 to 20 days has been devised. Rapid means of field identification of oral gram negative bacteriodaceae are being studied. The early wound healing process in the presence of the above organisms is also being investigated. Studies also are continuing on the microbiota to be found between oral surgical wounds and wound dressings. Data have been obtained which suggest the possibility of druginduced stimulation of immunologic protective mechanisms in oral tissues. The role of phagocytic dysfunction in chronic oral infections is under investigations.

Identification and Control of Oro-facial Infections of Military Importance

Comparison of Micromethod Systems with Conventional Media for Identification of Oral Streptococci

We have previously reported that a cost effective, portable rapid, labor saving micromethod for the identification of oral streptococci is as accurate as conventional methods, yet can be performed in half the time at one-fifth the cost. We have extended our study to include over 65 strains of oral streptococci including 8 strains of the microaerophilic S. anginosus-constellatus. The epidemiology of streptococcal infections especially in contaminated wounds has previously been neglected due to the large battery of tests necessary for identification to the species level. Although it is known that the majority of brain abscesses are caused by streptococci, the true incidence of each species involved in these abscesses is unknown. Trauma to the oral cavity, with its abundance and variety of streptococci, has recently been implicated as playing a larger role than previously appreciated. The micromethod has provided an improved epidemiologic tool for examination of this problem by providing clues as to the origin of the infection. The role of streptococci originating from oral combat type wounds is being investigated.

Microbial Contamination of Anesthetic Machines

Post-anesthesia pulmonary infections are primarily associated with equipment which permits rebreathing of gases by the patient. The aim of our study was to determine the extent of post-anesthesia microbial contamination of non-rebreathing anesthetic machines such as used in the

combat situation and which are presently in use for administration of $\mathrm{N}_2\mathrm{O-O}_2$ inhalation sedation, and to evaluate the effectiveness of the commonly used alcohol swab disinfection of the masal hoods. Cotton swabs moistened in sterile saline were used to obtain bacteriological cultures of the nasal hoods, corrugated breathing tubes, breathing bag, and gas outlet in the area of the non-rebreathing valve of nine Fraser Sweatman Quantiflex MDM Anesthetic Machines. The cultures were obtained (1) after the use of machines on each of 195 patients, and (2) after "disinfection" of the nasal hoods with alcohol. The results have shown that following the inhalation sedation no microbial contamination of the machine components proximal to the masks occurred. The extent of contamination of the masks differed, with counts of 0 to TMC (too many to count) colony forming units. Alcohol swabbing of anesthetic masks did not consistently eliminate bacteria as evidenced by the finding that more than 70% of 195 mask cultures yielded bacterial growth. Such a failure of sterilization may cause post-anesthesia pulmonary infections particularly in compromised patients. We autoclaved the anesthetic masks used in this study 50 times without any indication of mask deterioration. Autoclave sterilization of autoclavable anesthetic masks is recommended for Army use.

Microbial Contamination of Dental Units and Ultrasonic Scalers

In a recent study of the microbial contamination of the water system in 12 dental units and 9 ultrasonic scalers at two Army dental clinics we found that water samples from ultrasonic scalers, high-speed handpieces, and water syringe lines contained up to 2.6 million, 3.3 million, and 190,000 colony forming units/ml, respectively. Flushing the lines for two minutes reduced the microbial levels, but complete elimination of all bacteria was not achieved.

In another study we also found that the use of sterile disposable membrane filters attached to dental units can eliminate the microflora from the water of an ultrasonic scaler and high speed handpieces for up to 48 hours and 72 hours, respectively.

Surgical treatment of orofacial wounds frequently requires use of high-speed equipment and ultrasonic scalers.

Water from dental units has been shown to be frequently heavily contaminated with Pseudomonas spp. known to have a pathogenic potential, particularly in debilitated patients and those overtreated with broad spectrum antibiotics or corticosteriods.

Many war casualties must undergo prolonged antibiotic treatment, and it is known that their immune system is often compromised. These patients therefore are susceptible to infections, including wound infections, caused by microorganisms that are considered to be non-pathogenic to healthy individuals. It is, therefore, of utmost importance to assure that asepsis be maintained during emergency and routine treatment of military personnel and to eliminate any possibility that dental equipment may be a source of the infecting microorganisms.

In an attempt to control the microbial colonization of dental equipment, we designed and attached to dental units an iodination and filtration system.

The results indicated that the system failed to decontaminate the units. It appears that this failure was due to the dissipation of iodine from the water, or its binding to the copper tubings, or organic matter. Our preliminary results have also shown that sterilization of water lines in dental units and accessory equipment could be accomplished by replacing the residual water with alkaline gluteraldehyde (Cidex) for a period of 24 hours or less. Subsequent thorough flushing of units with filter sterilized water (15 minutes) to remove traces of the sterilant, and culturing of water samples, showed decontamination of units was accomplished. Ten days after routine use of the units the water from high speed handpieces, water syringes, ultrasonic scalers, and rinsing cup faucets remained bacteria-free. Twenty days later water from syringes became contaminated although water from other sources did not reveal microbial growth. These encouraging results warrant further testing and dissemination. Additional research is in progress.

The Rapid Serologic Identification of Oral Gram Negative Bacteriodaceae in Anaerobic Wound Infections

Progress on the isolation and characterization of previously reported electrophoretic differences in the antigenic components separating two strains of <u>Fusobacterium nucleatum</u> with electroimmunodiffusion and crossed electroimmunodiffusion has been unrewarding. It is hoped that other methods of separating the components of heterogeneous antigenic preparations, such as isoelectric focusing, will permit the isolation of specific antigens. These specific antigenic components will be utilized in the production of the monospecific antisera which will be basic to

the rapid field identification of bacteria in wound infections. Serological testing of the organisms under investigation has indicated that both hemagglutinating and complement fixing antibodies are produced to protein and polysaccharide antigens. In addition, it appears that species specific protein and polysaccharide antigens of a strain of <u>Fusobacterium nucleatum</u> can be isolated in the cell walls of the organisms. These findings suggest that rapid field identification of the organisms studied is possible in combat wounds.

A Serologic Investigation of Early Wound Healing Processes in the Presence of Oral Gram Negative Anaerobic Organisms

Having established a reliable assay for the consumption of complement via the alternative complement pathway in the guinea pig system, we are now using the complement consumption assay to examine the activation of human complement in both commercially obtained and leukapheresis obtained human sera. Cell wall preparations of <u>Fusobacterium nucleatum</u> type strains and of two <u>Leptotrichia buccalis</u> type strains have shown the ability to consume hemolytic complement activity in human sera without the apparent influence of reactive antibodies. The consumption of complement depended upon the concentration of cell walls in the assay and the temperature maintained during the incubation of sera with the cell walls. The nature of the complement consumption observed was consistent with that produced by other known activators of the alternative pathway. Information gained from additional progress in this investigation will be important to the

understanding of the pathogenesis of combat wounds infected with these anaerobic bacteria and also the progress of certain granulomatous diseases of man (osteomyelitis, periodontal diseases, etc.).

The Microbiota Between Surgical Wounds and Dressings

The bacterial contamination of wounds often occurs and results in delayed epithelial and connective tissue healing. Current attempts to identify the flora beneath the dressings of oral wounds, has centered on methods of disaggregation of bacteria from these wound surfaces. Recent data obtained from studies of disaggregation by sonication indicate that sonication has a killing effect on gram negative organisms, but increases disaggregation and cultivable numbers of gram negative bacteria if used at low power settings for extremely brief (≤ 5 sec.) treatment periods. Current studies using vortexing under anaerobic conditions for disaggregation, have isolated pathogenic organisms beneath the dressings of oral wounds. Impending studies will more closely identify the microbiota involved and the importance to healing in the oral cavity.

Immunoglobulin Levels in Inflamed and Normal Gingival Tissues

We have previously reported (Ann. Res. Prog. Rep. 1977) immunoglobulin levels of IgG, IgA, and IgM in 153 gingival tissue specimens consisting of normal, inflamed, and hyperplastic gingiva. The statistical analysis of the results has revealed additional information. The phenytoin hyperplastic and inflamed gingiva had a significantly

increased level of IgG when compared to the normal and idiopathic hyperplastic tissues. Only the inflammed gingiva had a significantly elevated IgA level. IgH was detected in 90% of the phenytoin hyperplastic and 71% of the idiopathic hyperplastic tissues, in contrast to only 40% of the normal and 42-47% of the inflamed tissue.

The immunoglobulin levels for IgG and IgA reflect the expected elevations due to the inflamed condition of the tissue as evidenced by the gingival index scores for each group examined.

The elevation in IgM for the phenytoin hyperplastic tissue does not reflect a correlation with the degree of inflammation and may possibly represent an immunoglobulin alteration which was drug-induced. Since the tissue immunoglobulin levels were assayed using both electroimmunodiffusion and radial immunodiffusion methodologies, the data for both methods was compared to determine the possible variability of results. The statistical analysis revealed no significant difference in the concentrations or standard errors of the two techniques used. This and similar studies of protective mechanisms following inflammation and infection may provide better understanding as well as means of prevention and treatment of acute and chronic infectious disease and wound infections encountered by the soldier in the field.

Phagocytic Dysfunction in Infectious Processes

High costs and the decreased effectiveness of military personnel occurs because of lost duty time during the delayed healing of maxillofacial wounds and postoperative infections.

Investigation has begun of impairments in neutrophil function. Trials of phagocytosis were completed using microscopic tests of phagocytosis. These proved inaccurate. Assays, using C¹⁴ labeled amino acids, are showing improved results and should allow comparisons of the kinetics of phagocytosis. Trials are being performed which will correlate phagocytic dysfunction with chronic oral infection. This will allow the development of a screening system for soldiers with neutrophilic defects and provide information for a more specific antibiotic therapy post trauma.

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- Setterstrom, J.A., D'Alessandro, S.M., and Gross, A.: A Comparison Between Single Radial Immunodiffusion and Electroimmunodiffusion For The Determination of Tissue Immunoglobulin Levels. Abstract submitted to IADR, for presentation March 1979.
- Gross, A., Setterstrom, J.A., D'Alessandro, S.M., and Van Swol, R.: Immunoglobulins in Periodontal Tissues. I. Concentrations of Immunoglobulins in Normal and Inflamed Gingiva. J. Periodontol. (Submitted for Publication).

Van Swol, R., Gross, A., Setterstrom, J.A., and D"Alessandro, S.M.: Immunoglobulins in Periodontal Tissues. II. Concentrations of Immunoglobulins in Granulation Tissue From Pockets of Periodontosis and Periodontitis Patients. J. Periodont. (Submitted for Publication).

Setterstrom, J.A., D'Alessandro, S.M., Gross, A., Godat, R.F., and Van Swol, R.: Immunoglobulins in Periodontal Tissues. III. Concentrations of Immunoglobulins in Dilantin Induced and Idiopathic Gingival Hyperplastic Tissue. J. Periodontol. (Submitted for Publication).

Gross, A. and Russell, E.A.: Microbial Contamination of Anesthetic Machines. Abstract submitted to IADR, March 1979.

Hawley, C.E. and Cooley, L.T.: The Anticomplementary Activity of Fusobacterium nucleatum in Human Sera. Abstract submitted to IADR, March 1979.

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- (U) Treatment Acceptance (U) Oral Health (U) Personality Factors
- 23 TECHNICAL OBJECTIVE. 24 APPROACH, 25 PROGRESS (Furnish Individual paragraphs identified by number. Procedo test of each with Jecurity Closethication Code.)
- 23. (U) Under Title X of the Military Code, the Army is authorized to routinely provide extensive and effective, quality dental treatment to the soldier. However, since the possibility of conflict is ever present, combat readiness is stressed via delivery systems which bring dentistry to the soldier to assure his oral state does not preclude performance of duty. This is different from the civilian system where the patient seeks treatment. It should be noted however, that no matter what delivery system is utilized it is neither comprehensive nor effective enough to assure compliance by the patient. Factors must be identified which will motivate the soldier to initiating, accepting, and maintaining dental treatment and thereby eliminating potential periods of ineffectiveness and lost duty time. These same factors will also enhance professional productivity and reduce greatly the treatment costs.
- 24. (U) Identify personality components unique to military patients which have a direct influence on treatment outcome. Identify which patients will respond to given methods of influencing preventive dental care. Identify those occupational factors which affect dental health care delivery by dental personnel. Identify those educational factors which lead to the best oral health care deliver.
- 25. (U) (77 10-78 10) No progress. Due to loss of principal investigator and our inability to replace him, this project is terminated.

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- (U) Osteoclast Activating Factor (U) Bone Resorption

 (U) Mononuclear Leukocytes (U) Concanavalin A

 23. TECHNICAL OBJECTIVE.® 24 APPROACH, 25. PROGRESS (Purnish Individual paragraphs identified by number. Proceeds total of each with Sociality Closeliteation Code.)
- 23. (U) To establish the precise role of human lymphocytes and macrophages in the generation of the bone resorber, Osteoclast Activating Factor (OAF), and also to determine characteristics of the generated OAF product.
- 24. (U) Functional relationships between subpopulations of human mononuclear leukocytes (Tand B-cells, and macrophages), with and without stimulation, will be systematically explored for their ability to produce OAF. Also, various physiochemical and immunological techniques as well as enzymes and inhibitors of known bone resorbing agents will be utilized to determine the specificity of OAF.
- 25. (U) (78 03-78 10) The change in principal investigator in October of 1977 necessitated restandardization of new investigators in the bioassay for bone resorption. OAF is being generated in mononuclear leukocyte cultures using concanavalin A. Purification of the generated OAF activity is now in progress. A correlary investigation into the biology of bone resorption involves supernatantsfrom anti-IgE stimulated basophil cultures which have demonstrated OAF-like activity on bone. Evidence was obtained which suggests that biodegradable ceramic placed in the vicinity of actively resorbing bone inhibits parathyroid stimulated release of 45Ca. This finding may indicate the use of ceramics to accelerate osseous wound healing.

Identification of Leukocyte Populations Responsible for the Production of Osteoclast Activating Factor and Their Role in Bone Resorption

Since the change in principal investigator on 1 October 1977, two investigators have been standardized in the bioassay for bone resorption using parathyroid hormone or freshly generated OAF as stimulants for 45Ca release, the biochemical and immunological methods utilized for the isolation and purification of OAF have been established in the Department of Microbiology, USAIDR, and the staffing of this NIDR/USAIDR interagency agreement is now complete with the arrival of a research chemist and a biological laboratory assistant. We have elected to generate OAF in mononuclear leukocyte cultures using concanavalin A (Con A) because this mitogen offers certain advantages to the subsequent steps in purification of OAF. Large volumes (500 - 1000 ml) of OAF activity have been produced with Con A, and the activity produced correlates well with that found in conventional cultures. The uptake of tritiated thymidine in these cultures does not correlate with the synthesis of OAF. We are now beginning the purification phases of the generated OAF activity. Other investigations into the biology of bone resorption include a collaborative study with NIDR in which the supernatants from anti-IgE stimulated basophil cultures have demonstrated OAF-like activity on bone. In our own laboratory, we have generaged evidence to suggest that biodegradable ceramics (tricalcium phosphate), when placed in the vicinity of actively resorbing bone, will inhibit the anticipated parathyroid hormone stimulated release of 45Ca.

This latter finding may be important to the use of ceramics to accelerate osseous wound healing. We have also investigated the effect of certain gram negative pathogens on bone resorption. Our data indicates that sonicate preparations of <u>Fusobacterium polymorphum</u> and <u>Leptotrichia buccalis</u> contain factors that will both inhibit and stimulate the resorption of bone <u>in vitro</u>. However, when the sonicates are tested in dilute concentrations, only the stimulatory effect is observed. Further studies in this area are indicated as it is important to understand the effect of these organisms on the dynamics of bone repair in contaminated wounds.

PUBLICATIONS

Hawley, C.E., Kakari, S., Wyan, V., and Lamb, L.: An Evaluation of Con A as a Stimulant for the Production of Osteoclast Activating Factor. Abstract submitted to IADR, March 1979.

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Foreign Intelligence Considered				MAME: Russell, E.A., COL, DC							

[PII Redacted]

- (U) Nitinol; (U) Shape Memory (U) Osseous Fixation

 (U) Fixation Devices: (U) Surgical Staples

 22. TECHNICAL OBJECTIVE. 24 APPROACH, 23. PROGRESS (Fumilah Individual paragrapha Identified by number Proceeds 1021 of each with Security Classification Code.)
- 23. (U) To exploit the shape memory phenomenon of 55-nitinol for enhancement of the treatment capabilities of the Army dentist and physician. Realization of the conceptual uses of this unusual metal will result in substantial savings of laboratory costs and professional man-hours.
- 24. (U) To design, fabricate, and test by means of animal and human studies, the following devices; (1) flexible wire clasps that will withstand accidental deformation out of the mouth, yet recover in the mouth; (2) prestressed surgical fixation staples and plates that will bend or contract slightly at body temperature, bringing bone fragments into close approximation or under slight compression; (3) self-anchoring fixation pins and endosseous implant devices; (4) collapsible devices for placement into defects (cyst cavity, cleft palate, etc) through orifices smaller than the inside diameter; (5) fixed and removable prosthetic appliances, restorations or precision attachments that can move into undercuts in the mouth.
- 25. (U) (77 10-78 10) A new generation of novel "force calibrated" surgical staples have been developed and applied successfully in the repair of osseous tissue. The advantages are simplified osseous fixation, elimination of adverse forces required with wiring techniques, reduced operating time, excellent toleration of staples by tissues and the consistent and continuous application of pressure to opposed surfaces for rapid healing.

Development and Evaluation of Nitinol For Use in Army Dentistry

A new generation of novel "force-calibrated" surgical staples has been developed and used with a high degree of success in the repair of osseous wounds. Nitinol staples with a diameter of .075 inches were used to stabilize elevated alveolar segments in fiftcen dogs. The elevated segment of alveolar bone was pedicled off a lingual mucoperiosteal flap. Holes were made with a twist drill in the elevated alveolar segment and basal bone near the vertical osteotomy sites. Grafts of different types were interposed between the elevated alveolar segment and basal bone. These nitinol staples, constructed in such a manner as to produce compression, were then inserted in the prepared holes thus achieving fixation of the alveolar segment.

The design of the nitinol staples permitted us to achieve fixation without stripping the alveolar segment of its vital blood supply.

The staples maintained fixation of these elevated alveolar segments even in those cases in which a biodegradable graft was sequestrated.

The major advantage of the nitinol staples over intraosseous stainless steel wires were as follows:

- 1) The nitinol staple permitted satisfactory intraosseous fixation without necessitating stripping of the mucoperiosteum from the pedicled alveolar segment.
- 2) The nitinol staples were easy to place and did not necessitate the application of adverse forces on the pedicled segment as seen in

tightening of intraosseous stainless steel wire.

- 3) The ease of placement of the nitinol staples markedly reduced operating times.
- 4) The nitinol staples were well tolerated and did not become exposed to the oral cavity as was seen in a similar study in which stainless steel wires were used to achieve fixation.
- 5) The staples apply continuous pressure to the opposed surfaces and therefore allow for faster healing.

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NAME. US Army Institute of Dental Research

ADDRESS: Wahsington, DC 20012

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Washington, DC 20012

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Brunner, D.G., LTC, DC

22. KEYWORDS (Fracedo EACH with Security Classification Code) (U) Dento-Facial Injuries (U) Noise Hazards

(U) Oral Health (U) Spoon-Toothbrush
23. TECHNICAL OBJECTIVE. 24 APPROACH, 25. PROGRESS (Furnish Individual peregraphs Identified by number. Proceeds text of each with Society Classification Code.)

- 23. (U) To develop new and simplified methods of preventing oral diseases and maxillo-facial injuries. To assess new methods of (1) improving the biologic management of militarily relevant oral conditions and (2) improving the cost-effectiveness factors of preventive dental therapy in the military.
- 24. (U) Studies will be conducted on military installations which will evaluate (1) methods of preventior of militarily relevant abnormalities and maxillofacial injuries; (2) methods of improving preventive dentistry delivery systems; (3) methods of improving cost-benefit ratios concerning delivery of preventive dentistry as a consequence of military duty; and (4) investigate the various hazards involved in the Army dental health delivery system.
- 25. (U) (77 10-78 10) Additional data obtained in a survey of dentofacial injuries among Army personnel is consistent with previous data reported. A method for rapid evaluation of troop oral health has been developed. Work on the development of a spoon-toothbrush combination for field use is continuing. Continuation of a study on noise levels in Army dental clinics and laboratories is consistent with previous data showing unacceptable noise levels in many cases. A study of the cleansing efficacy of a polyester toothbrush used by the soldier has not indicated any advantages over the nylon toothbrush. This study is continuing.

Aveilable to contractors upon originator's approval

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RESPONSIBLE INDIVIDUAL

21. GENERAL USE

NAME: Cutright, D.E., COL, DC

Foreign Intelligence Considered

TELEPHONE: 202-576-3484

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Preventive Dentistry Measures of Military Significance

A Epidemiological Survey of Accidental Dento-Facial Injuries Incurred Among Active Duty Army Personnel

Two hundred additional accidents have been studied beyond the 747 analyzed in the FY77 annual report. The added data continues to show that close to two thirds of the accidents occur in the age group 17-20 year among individuals with less than one year of service. Over onethird of the individuals have the military rank of E-1. Sport related accidents, most of which were "combat" versions of football and basketball sponsored by the military accounted for 17.5 percent of the accidents as opposed to the 20.3 percent perviously reported. Fist fights continue to account for just under 30 percent of the accidents. It was interesting to note in the most recent data that none of the accident victims used mouth guards in sport situations while in the previously reported data only 3.3 percent of the injured used mouth guards. The conclusions reached on the previously reported data still obtain, namely that a significant amount of treatment time is expended by the army dental corps on the injuries studied and that it seems likely that a satisfactory cost-benefit ratio might be achieved by the fabrication and mandatory use of mouth guards by personnel participating in Army sponsored contact sports.

A Rapid Screening Test for Evaluating Troop Oral Health

Gingival inflammation and alveolar bone loss are the main cause of tooth loss in military patients over age 35. It has been shown that gingival inflammation frequently starts at an early age and requires extensive time and expense to treat in military personnel during their career.

A simple, objective, and inexpensive clinical test for gingival inflammation would permit rapid screening examination of soldiers for the presence of initial gingival inflammation and more severe bone loss. Those soldiers giving positive results could then be identified and thoroughly examined to determine the extent of pathology present and determine the preventive type treatment required.

Such a test should provide reliable and valid diagnostic results with a minimum expenditure of time, materials, and personnel resources. Fluctuations with regard to subjective examiner error would be kept to a minimum. The object of this study was to devise such a test.

Patients receiving Oral Hygiene Instruction (OHI) at an established dental clinic were utilized for this study. Subjects were tested before, during and after the OHI series. Each subject was examined for gingival inflammation using the Gingival Index (G.I.) of Loe and Silness, to determine which group the subject belonged to so that correlations with other parameters could be done. At the start of the study a two minute salivary sample was collected from the subject in screw-top scintillation vials and the volume was recorded. The sample was immediately tested using a Hema-Combi-Stix (HCS) to assay for the presence of blood and protein. Each subject was then asked to brush his teeth for one minute using the standard issue tooth brush which had been wet with plain water. After brushing, the subject rinsed with 20 ml of distilled water, which was collected for analysis in a labeled scintillation vial. The tooth brush itself was rinsed in 10 ml distilled water in a separate scintillation vial. The water in both vials was tested for hemoglobin and protein using the (HCS). The samples were then

frozen for laboratory analysis. Quantitative hemoglobin and protein levels were determined on all samples for comparison with the HCS and G.I. results in order to determine if hemoglobin and protein levels using HCS is a valid test for gingival inflammation.

The following conclusions were drawn:

- 1. The Hema-Combi-Stix may not be sensitive enough to differentiate gradual or slight changes in severity of inflammation.
- 2. A decrease in protein found in the toothbrush rinse after oral hygiene instruction is associated with a reduction in Gingival Index.
- 3. The best correlation was made between Gingival Index and hemoglobin content of toothbrush rinses, and between Gingival Index and the protein content of tooth brush rinses.
- 4. Accordingly, the detection of hemoglobin traces on the toothbrush by the cyanmethemoglobin method can be used as a rapid screening test for gingival inflammation.

The incorporation of this data into the Army Dental Care Delivery system will decrease oral disease and prevent lost duty time required for treatment.

Development, test and Evaluation of a Spoon-Toothbrush Combination for Field Use

Testing of a prototype spoon-toothbrush combination by troops in the field showed that 93% of the individuals responding to a questionnaire indicated they would use the device if it was made available to them.

Based on this positive response, final plans were completed for the design of a spoon-toothbrush combination for a single use by field troops.

A contract was awarded for the tooling and mold production and 50 sample

parts. A request for patent right determination has been completed and is awaiting action.

Phase II of this study will consist of field tests and evaluation of the effects of the spoon-toothbrush on the oral health of the individuals using it.

An Epidemiological Survey of Noise Levels in U.S. Army Dental Clinics and Laboratories

This study which surveyed six clinics at two posts showed dental handpieces which had peak noise levels over 97 dB. Model trimmers were also found to produce levels of between 90 and 95 dB. These ranges are potentially damaging and reinforced the need to monitor all such equipment for acceptable noise levels. A small relatively inexpensive device is currently available which could be purchased by individual clinics for such use. This would produce the following. One, it could monitor new turbines when placed into use to determine if they are defective as received; second, this would serve as an internal check on first echelon maintenance of handpieces, and third, this would alert personnel to unacceptable noise levels.

A phase of this study currently being considered is a retrospective analysis of hearing acuity as determined on annual physical examinations for those specialties such as crown and bridge where extensive handpiece use is necessary.

Evaluation of the Cleansing Efficacy of a Polyester-Bristled Toothbrush

In Phase I of this study, no statistically significant differences in cleaning efficacy were noted between a standard nylon bristled brush and either a ribbed or plain polyester bristled brush. A reduction in plaque of 40.8% was noted for the nylon brush, 34.5% for the plain polyester and 35% for the ribbed polyester brush. The next phase of this study will examine the differences in cleansing efficacy following exposure to moisture. The importance of the polyester brush to the combat soldier is that it does not absorb water, is easily dried and therefore does not support bacterial growth.

PUBLICATIONS

Lyon, T.C., Brunner, D.G. and Rathke, J.R.: A Survey of Noise Levels Generated by Dental Equipment. J. Dent. Res. <u>57</u>, 288, 1977.

Katz, R.V., Lyon, T.C., Brunner, D.G. and Barnes, G: The Pathology of Dentofacial Injuries: Epidemiology and Economics. J. Dent. Res. 57, 219, 1977.

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(U) Neodymium Laser; (U) Carbon Dioxide Laser; (U) Laser Welding; (U) Laser Sterilization; Laser Effects On Soft Tissue 23. TECHNICAL OBJECTIVE. 4 24. APPROACH, 25. PROGRESS (Furnish Individual paragraphs identified

- 23. (U) To determine the feasibility of the application of laser technology to prost thetic rehabilitation and to maxillofacial wound debridement and treatment.
- 24. (U) Energy levels, methods of contour and approximation of pontics to establish optimum weld patterns and strengths will be investigated. This will be accomplished first in a bench set-up and secondly in animals to establish feasibility and safety. Comparison of the response of different mucosal sites to ${
 m CO}_2$ laser energy will be made. Evaluation of the response of bone to the CO_2 laser will be made. Laser vs. surgical debridement of contaminated wounds will be done. Laser sterilization of metal instruments will be evaluated.
- 25. (U) (77 10-78 10) Work completed to data in Rhesus monkeys indicates that in-vivo laser welding of fixed prostodontic appliances is possible without any observable effect on the dental pulp. The potential of the CO₂ laser in oral soft tissue wound debridement has been demonstrated. Studies on the use of the CO2 laser for the sterilization of small dental instruments have been complet a. Sterilization was rapid and consistent No deleterious effects were noted on cutting edges. A study of the effect of laser radiation on bone is in progress.

Application of Laser Technology to Military Dentistry

Effects of Carbon Dioxide Laser Radiation on Oral Soft
Tissues: An Initial Report

Three different oral mucosal sites were exposed to the Carbon Dioxide surgical laser. Although nearly every laser exposure caused the loss of the epithelium and penetration of the lamina propria up to 0.5 mm., no bleeding occurred. HIstologic changes included shredding of keratin, vacuole formation and basal cell degeneration in the epithelium. Glassy homogenization of collagen and vascular occlusion characterized the alterations in the connective tissue. These changes were consistent for each of the three tissues examined. Within the limits of the experimental procedure, it appears that the laser vaporizes an additional 0.1 mm. of tissue for each 5 watt increase in power when the time of exposure remains constant. These qualities of the ${\rm CO}_2$ Laser show it's potential as a wound debridement methodology as follows:

- 1. It sterilizes surfaces.
- 2. Provides immediate hemostasis.
- 3. Increases the rate of healing.
- 4. Allows precise depth of tissue removal.
- 5. Is easily utilized in a surgical suite.
- 6. There is no patient contact.

A New Method of Sterilization: The Carbon Dioxide Laser

The CO_2 laser is reflected by metal surfaces and is virtually completely absorbed by biologic tissues resulting in their vaporization. In view of these facts, it seems possible that the CO_2 laser could be used

as a means of sterilizing small metal objects such as implants, endodontic instruments or periodontal scalers.

Development of proper, effective and improved means of sterilization of various medical and dental instruments and materials is of continous interest to the military. In the dental profession, the dulling of instruments after autoclaving is a problem requiring many man hours spent on resharpening. Improper sterilization procedures often cause deterioration of expensive instruments necessitating their frequent replacement. The idea of using the CO₂ laser for sterilization is a completely new approach, and its feasibility should be investigated. If successful, such a method may have wide application in other fields of medicine.

Twenty scalpel blades contaminated with bacterial spores were exposed to the laser and subsequently cultured. In addition, six blades contaminated with the spores but not exposed to the laser served as controls.

None of the laser exposed blades showed any evidence of growth of the microorganisms. However, all of the control cultures contained abundant growth of the spores. Examination with the SEM revealed no deterioration of the blade edge.

These results demonstrate that the Carbon Dioxide laser is capable of sterilizing metal instruments. It's application in the surgical suite is being pursued.

Effect of Carbon Dioxide Laser on Periodontal Scalers

Previous work in our laboratory has demonstrated that metal instruments may be sterilized by the use of the carbon dioxide (CO_2) laser. However,

it has not been established whether the laser exposure used for sterilization would alter the cutting edge of dental instruments. Therefore, the purpose of this study was to determine whether CO2 laser radiation has any deliterious effect on the cutting edge of periodontal scalers.

Twenty-two randomly selected Morse No. 1 & No. 3 Scalers were divided into two groups. The laser exposed group (LEG), 14 scalers, was exposed to the $\rm CO_2$ laser at 15 watts for 20 seconds. The control group (CG), 8 scalers, was not exposed to the laser.

After laser exposure, both groups of instruments were used to scale the root surfaces of extracted human teeth above the clinical epithelial attachment until dullness was obtained. Dullness was determined by the fingernail test.

Subsequently, all the instrument cutting edges were examined by the scanning electron microscope (SEM). SEM micrographs of both control and experimental cutting edges were compared by two examiners to determine if any difference in edge wear obtained between the two groups.

Comparison of the SEM micrographs demonstrates that there is no difference in the edge wear pattern between the LEG and the CG. Since both groups were subjected to essentially the same stresses, it appears that ${\rm CO_2}$ laser exposure of the cutting edges does not have a detrimental effect on the edges of the scalers.

The results of this study strongly suggest that use of the ${\rm CO_2}$ laser for sterilization of metal instruments (periodontal scalers) will have no effect on the clinical performance of the scaler.

Bone Response to Carbon Dioxide Laser Radiation

Recent studies have suggested that the CO_2 laser is of potential value in orthopedic surgery for the treatment of osteomyelitis, debridement and biopsy of malignant tumors. Findings from these studies have generated the need for investigations of the effects of CO_2 laser radiation on bone to include the extent of injury and the dynamics of regeneration.

Combat injuries are characterized by their incidence of contamination resulting in a requirement for lengthy and meticulous debridement. The CO₂ laser has unique properties which make it a potentially valuable instrument in the surgical treatment of combat-induced wounds.

The high incidence of infection of combat wounds contributes to operative and postoperative complications. With the CO_2 laser, infection at the operative site is not a contraindication to surgery. Vaporization of the infected material of contaminated wounds, including osteomyelitis, is possible and could result in a sterilized site. The obvious advantage is fewer postoperative infections, and consequently a decreased need for long-term, high-dose antibiotic therapy. Hospitalization time would also be reduced.

A study is in progress using the surgically exposed rat tibia as an in-vivo model. Its purpose is to investigate the histological response of bone to a sustained impact of radiation delivered from a carbon dioxide surgical laser. The results have not yet been analyzed.

Use of the ${\rm CO_2}$ Laser in Sterilization of Endodontic Reamers

The need exists for a rapid chairside technique of sterilizing endodontic instruments because endodontic instruments are quickly contaminated by contact with the oral flora and also by digital manipulation. Currently the only rapid chairside sterilization technique is the salt (glass bead) sterilizer which unfortunately is not consistently reliable. The development of an effective rapid chairside sterilization technique for endodontic instruments will aid in decreasing the failure rate of endodontic procedures. The use of the ${\rm CO}_2$ laser system also has the potential to greatly decrease chairtime per procedure, thereby improving the efficiency of the endodontist.

The purpose of the present investigation is to determine if endodontic reamers contaminated with known microorganisms can be effectively sterilized using a $\rm CO_2$ laser.

Fifty endodontic stainless steel reamers were contaminated with bacterial spores and randomly divided into two equal groups (experimental and control).

The experimental group was exposed to the laser and cultured in thioglycollate media. The control group was also cultured.

Preliminary results indicate that the laser is completely effective in sterilizing the endodontic instruments.

In Vivo Laser Welding

Full crown preparations of the maxillary incisor teeth of two Rhesus monkeys were made following partial gingivectomies. Gold crowns were fabricated. Half of the crowns (right lateral and central incisors) were

laser welded on the laboratory cast before insertion into the animals. These crowns served as controls. The other crowns (left central and laterals) were placed on the preparations in the monkeys' mouths and laser welded in vivo. After five days all the teeth were removed and the pulps were examined histologically. The results of this short-term follow-up indicate that the dental pulp is not injured by in vivo laser welding.

Three additional animals are currently being prepared for study in a similar manner but the follow-up in two of them will not be finalized for three months.

PUBLICATIONS

Adrian, J.C., Effects of CO Laser Radiation on Oral Soft Tissue: An Initial Report. Mil. 2 Med. (IN PRESS)

Adrian, J.C. and Gross A., A New Method of Sterilization: The Carbon Dioxide Laser. J. Oral Path. (IN PRESS)

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- (U) Pulp tester (U) Amalgam Alloys (U) Crown and Bridge

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 TECHNICAL OBJECTIVE.* 24 APPROACH, 25 PROGRESS (Purnish Individual paragraphs Identified by number, proceeds test of each field goodwift Classification Code.)
- 23. (U) To evaluate new materials and material of special interest to the Army dentist. Criteria for selection of materials, devices or techniques for evaluation are based on anticipated high potentials for: (1) savings of fiscal and/or manpower resources; (2) work simplification; (3) improved health care delivery in combat areas; and (4) enhanced safety with respect to professional and ancillary personnel as well as to the patient.
- 24. (U) New materials will be evaluated on the basis of the following parameters; Composition, microstructure, physical and mechanical properties, cytotoxicity, and clinical performance.
- 25. (U) (77 10-78 10) Three "low-gold" alloys tested appear to have potential for Army use. The testing of a number of new adhesive restoratives has not revealed any that are acceptable for more than very limited use in the field. In-vivo testing of high-copper amalgams and base metal crown and bridge alloys for adverse tissue reactions is in progress. Clinical testing of the USAIDR developed pulp vitality tester has begun. A novel apparatus for testing flow characteristics of dental materials has been developed. A new casting technique evaluated for potential field use has been found unacceptable. Stress relaxation characteristics of 4 temporary restoratives have been studied. The efficacy of an electroless plating system for caries prevention is being studied in experimental animals. Twelve elastomeric impression materials have been found acceptable for Army use.

silable to contractors upon originator's approval

Development and Evaluation of Dental Materials and Materiel for Army Use.

Laboratory Evaluation of "Low-Gold" Casting Alloys

Compositions, structures, mechanical properties and handling characteristics of three relatively inexpensive gold-containing casting alloys have been studied. Test materials included Midas (J.F. Jelenko, Inc.), Neycast (J.M. Ney Co.), and Minigold (Williams Gold Refining Co.). Gold content of the "economy" materials ranged from 40 percent (Minigold) to 47 percent (Midas). Alloying elements common to the three materials included silver, palladium and copper. Castings of the test-materials exhibited a distinct biphasic structure. Heat-treatment characteristics and values for hardness, strength, regidity and ductility were comparable to those of conventional (71 to 80 percent gold containing) type III dental alloys. User tests showed that Midas, Neycast and Minigold can be cast, machine-finished and polished through the use of simple and routine laboratory procedures. The three "low-golds" appear suitable for long-term clinical evaluation.

Definitive demonstration of the efficiacy of these materials in field dental practice could provide impetus for the expanded use of relatively inexpensive casting alloys throughout the entire Army dental health care delivery system.

Feasibility Study and Development of An Adhesive to Dentin and to Tooth Enamel

Development of reliable adhesive composite restoratives would increase the longevity of esthetic dental restorations and enhance the effectiveness

of pit and fissure sealants in the prevention of dental caries.

Characterization studies on graft initiators, composite restoratives and adhesive supplied by a contractor have been conducted. The recently developed products are merely state-of-the-art reflections of commercially available dental restorative systems.

Products developed and submitted by the contractor will be evaluated for assessment of their potential usefulness in field dental practice as they become available.

Tissue Response to Dental Amalgam Alloys

Subcutaneous implants of the corrosion products of high-copper containing dental amalgam alloys have been linked to the production of severe abscesses. Sufficient data for extrapolation of these findings to human experience are not available presently.

Spontaneous avulsion of implants of the corrosion products of certain high-copper containing dental amalgam alloys (Tytin and Optalloy II) has necessitated redesign of the subject study. Loss of the implants occurred prior to the first scheduled tissue-harvest (3 months post surgical). Similar findings for other copper containing material have been reported by other investigators. The phenomenon may be a manifestation of early and severe abscess formation. The work will be repeated to allow observation of Tytin and Optalloy II-implants at weekly intervals for a period of 3 months.

Information gained from such studies is essential to the rational prevention of adverse material-tissue interactions that may restrict the combat effectiveness of the field soldier.

Development of a Pulp Vitality Tester

Clinical Testing of the USAIDR optical vitalometer was initiated. To date, electronic noise has prevented accurate determination of the frequency and amplitude of pulsatile signals received from vital teeth. The problem would appear to be a manifestation of the random scattering and diffusion of multichromatic light within the crystalline components of the test teeth.

Extrusion Rheometry of Fluid Materials

A capillary extrusion rheometer was designed, built and tested using Newtonian oils of known viscosity and seven endodontic sealers. The novel apparatus and technique appear highly useful for comparative assessment of the flow characteristics of a variety of fluid materials available for use in military dental practice. It is anticipated that the new laboratory equipment and technique will play prominent roles in the development of fluid-based polymeric materials for the immediate field treatment of combat inflicted maxillofacial injuries.

Evaluation of a New Casting Technique

The performance potential of a vacuum-pressure casting machine

(Automatic Chronomatic System II, Whaledent International) has been evaluated. Small dental castings fabricated from silver-rich high fusing alloys (Neydium, CAmeo and JPW) exhibited severe macroscopic and microscopic porosity. On the other hand, use of the machine for the casting of a silver-free material (Olympia) yielded structurally sound castings. At

the present time, lack of versatility contraindicates routine use of the vacuum-pressure device in field dental practice. This information is being disseminated to the field.

Feasibility of the Use of Solid Photography in the Fabrication of Fixed Dental Restorations

Development of a reliable computerized precision milling process would make possible the use of inexpensive and nonstrategic metals and alloys for the fabrication of fixed restorations.

Wax patterns and cast full crowns have been fabricated and transmitted to the prime contractor (Solid Photography, Inc.) for replication by a novel precision milling procedure.

Support of the subject contract research will be rendered as required.

Stress Relaxation of Interim Restoratives

Stress relaxation behavior cannot be used as the sole criterion for selection of a temporary restorative for emergency field use. However, realization of the degree to which relaxation may occur is essential to the rational temporary treatment of traumatized and diseased teeth.

Stress relaxation of four temporary restoratives was studied. In the vicinity of ambient mouth temperature, the relaxation characteristics of an unmodified zinc oxide-eugenol cement were more favorable than those of IRM and Cavit. The plastic behavior of gutta-percha temporary stopping precluded assessment of its relaxation at temperatures in excess of 22°C.

Determination of the Efficacy of Electroless Plating in Caries Prevention

Development of effective and reliable materials for the prevention of dental caries will reduce substantially the extent to which professional dental services to the combat soldier must be rendered.

Silver has been deposited by a redox reaction on the occlusal and smooth surfaces of the maxillary molars of young caries-susceptible rats. Observation of treated and nontreated teeth will continue until the effectiveness of electroless plating in the prevention of dental caries can be established. This project continues to have a high potential for military usage in reducing the evidence of gingivitis and preventing lost time required for treatment of dental disease.

Evaluation and Improvement of New Adhesive Restoratives

Laboratory characterization of four proprietary products (Aspa, Powderlite, Cervident and Adaptic) and one experimental formulation (PRCA) has been completed. Significant differences in neither the strengths nor the adhesive qualities of the test materials were found. In general, adhesion of the four test materials to the components of tooth structure was unreliable.

The ultimate aim of the composite adhesive research program is to identify and to develop materials that can be used noninvasively by combat field medics for the immediate and functional restoration of damaged teeth. Presently, however, field application of the available dental adhesive restoratives should be limited solely to the restoration of conventional Class III and Class V cavities.

Assessment of the Tissue Response to Base Metal Crown-and-Bridge Alloys

Rat tissues affiliated with one-year subcutaneous implants of the corrosion products of two nickel-chromium alloys (Ticon and Gemini II) and an iron-chromium based material (Dentillium CB) have been harvested. Findings from the gross inspection of the tissue segments controvert the significance of the previously observed adverse responses of in vitro cell cultures to the test materials. Microscopic examination of the tissues is in progress.

Data from the present investigation will be used in the establishment of rational guide lines for the study of tissue responses elicited by all casting alloys used within the Army dental health care delivery system. From the standpoint of patient safety, the most suitable materials for military application will be identified.

Development and Evaluation of Elastomeric Impression Materials

The clinically relevant properties of five polysulfide, seven silicone and two polyether impression materials were studied. The materials were tested to allow assessment of mixing time, working time, consistency, permanent deformation, dimensional stability, flow, strain in compression, and ability to reproduce fine surface detail. All test materials were found to be acceptable for military use. Comparatively, however, the polyethers (Impregum and Polyjel) exhibited exceptional accuracy. A putty (Optosil)-silicone wash (Xantopren) system showed inordinate ability to counter-replicate fine surface detail.

Additional Research to make possible the use of elastomeric impression materials under the adverse conditions of field storage is now in progress.

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- (U) Base Metal Restorations (U) Base Metal Casting
- 23. (U) Annual Army expenditures for precious metals utilized in the fabrication of fixed dental prostheses are in the vicinity of \$1,000,000. The cost of an equal volume of base metal alloy is \$30,000. Properties of base metal alloys indicates however, that these alloys cannot be utilized for small castings without drastic metallurgical modifications. This work is therefore being conducted to: (a) Develop heat treatment methods for controlling properties of nickel-chromium based casting alloys; (b) Evaluate nickel-chromium based alloys for use in operative dentistry.
- 24. (U) The properties of nickel-chromium based alloys will be studied in details by various physical methods available in order to devise procedures which will optimize their usefulness. Any improvement obtained will be evaluated clinically.
- 25. (U) (77 10-78 10) The influence of investment material and technique on the fit of base metal restorations was assessed. Two investment materials, "Ceramigold" and "Complete" were studied. Castings were made in ticon, Ceramalloy, NP-2 and victory. A total of 384 full crowns were cast. Only 12 percent were considered adequate. Twenty-six percent were oversized and 62 percent were undersized. The findings indicate a need for improved accuracy in the techniques provided with the above investment materials.

Development and Improvement of Metallic Restorative Materials

The influence of investment material and technique on the fit of base metal restorations was assessed. Investments included Ceramigold (Whip-Mix) and Complete (J.F. Jelenko). Design of the study allowed comparative evaluation of (1) the accuracy of castings made by hygroscopic and thermal techniques; (2) the effects induced by dilution of the investment materials liquid components; and (3) the accuracy of castings made with and without the use of asbestos-lined steel rings. Accordingly, 384 full crown wax patterns were constructed on stone dies and invested. Burnout of the molds was accomplished as prescribed by the respective manufacturers of the investments. An equal number of molds made from each material were cast in Ticon, Ceramalloy, NP-2 and Victory. The castings were placed on their respective dies, examined with the aid of a stereoscopic microscope and scored as adequate (x), oversize (+) and undersize (-). Overall, fit of the test castings was poor. Individual alloy-investment interaction appeared significant. Ceramalloy and Ticon were the only alloys from which acceptable restorations were cast. Scores for the total sample population were distributed as follows: (x) 12%; (+) 26%; (-) 62%. With Complete, castings of NP-2 and Victory were consistently under-size. However, castings of all test alloys produced in ringless, hygroscopically expanded Ceramigold (powder and undiluted liquid) molds were oversize. The findings suggest that further modification of the Ceramigold hygroscopic ringless technique may enhance the accuracy of cast base metal restorations.

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 12. KEYWORDS (Freedo EACH with Security Closelfheetten Code)

 (U) Lip Pathology; (U) Tongue Circulation; (U) Dimethyl

 Sulfoxide: (U) Adhesives: (U) Oral Diagnosis: (U) Maxillary Ameloblastoma

 23. TECHNICAL OBJECTIVE. 24 APPROACH. 25. PROGRESS (Furnish Individual paragraphs Identified by number. Precedo test of each with Security Closelfheetten Code.)

 23. (U) To recognize, characterize and develop effective therapeutic measures for those lesions and conditions which effect the soldier due to military duty. The recognition of environmental and other factors which participate in the etiology of lesions and conditions unique to the military or are causally related to military duty will enable the development of interceptive or therapeutic measures.
- 24.(U) To detect through clinical and/or microscopic observation oral lesions or a condition unique to the military population. To identify oral lesions or conditions which, though not unique to the soldier, are etiologically related to the performance of duty. Once identified the natural history including etiology, therapy, and prognosis will be established utilizing appropriate methods such as surveys, animal, and human investigations.
- 25. (U) (77 10-78 10) An evaluation of the effectiveness of actinic blocking agents used by the Army is in progress. An analysis was made of 24 cases of maxillary ameloblastoma. A study of collateral circulation in traumatic injury of the tongue was completed. Results show that collateral circulation will maintain the health of tongue tissue when one of the common carotids is ligated. The oral manifestations of diagnostic value in 21 tropical diseases are being catalogued. A study of instrument-free topical administration of lidocaine with DMSO suggests that anesthesia can be obtained during cavity preparation by such an approach. A study is in progress on means of minimizing periodontal problems following third molar removal.

Natural History of Oral Lesions

An Evaluation of the Effectiveness of Actinic Blocking Agents

The purpose of the project was to evaluate lipscreening agents issued to U.S. Army Troops for their effectiveness in preventing sunburn. "Hairless" rats were selected as the test model to eliminate the need for depilatory techniques. However it has been determined that "hairless" rats do not show an erythematous reaction to actinic radiation of their skin. Furthermore, white albino rats shaved and exposed to actinic radiation were found also to yield negative results. At present, the project cannot be completed without an animal model that will yield a visible erythematous reaction on exposure to actinic radiation. A guinea pig, rabbit and mouse will be tested in the future. If none of these models yield positive results, skin samples will be obtained from each animal and examined histologically and biochemically to determine if there is a reason for this apparent resistance.

Soft Tissue Response to Selected Commercial Adhesives

Frequently military patients attempt to self-repair their prosthetic appliances with adhesives not specifically designed for intraoral use. The purpose of this study was to investigate the histological response of soft tissues in contact with three commercially available adhesives (a methyl-2-cyanoacrylate compound, an epoxy resin-resin amine compound and a butyl acetate cement) implanted in their polymerized or solidified state. Histologic evaluation has been completed during FY78.

Of the three adhesives evaluated, only the methyl-2-cyanoacrylate compound appeared to cause a significant soft tissue response adjacent to the implants. Because this compound possesses the ability to induce a moderate to severe inflammatory response in the soft tissue, it is felt that methyl-2-cyanoacrylate adhesives should be included as a possible etiological factor when evaluating self-repair denture irritation of the oral mucosa.

The Maxillary Ameloblastoma: An Analysis of Twenty-Four Cases

The maxillary ameloblastoma presents clinically as a potentially more dangerous lesion than its mandibular counterpart. The microscopic appearance of this locally aggressive neoplasm challenges the diagnostic acumen of the pathologist and its variable biologic behavior dictates a radical therapeutic approach by the surgeon. The computerized files of the Armed Forces Institute of Pathology and the U.S. Army Institute of Dental Research were systematically searched for all cases coded as ameloblastoma of the maxillae. The material included cases from the military, Veterans Administration, and civilian sources. Twenty-four cases of histologically verified maxillary ameloblastoma were found and follow-up information was received on a total of 16 from various treatment centers. A questionnaire was used in each case to ascertain age, sex, race, anatomic site, treatment modality, and recurrence. Clinical findings demonstrated an average age of 45.6 years, a male/female ratio of 2.4:1, and an equal racial distribution between whites and blacks. Eighty percent of the

tumors occurred distal to the maxillary cuspid. A slowly enlarging mass was the primary clinical sign in over 90% of the cases. Recurrences were noted in 8 of the 16 cases. The majority of the tumors demonstrated a mixed follicular pattern. Therapy should consist of either surgical excision or hemimaxillectomy. The surgeon must carefully weigh the potential danger of the neoplasm against the deformity and disability caused by the surgical procedure.

The Microvasculature of Induced Collateral Circulation of the Tongue

Previous studies have contained little substantial evidence relating to the microvascular effects induced by unilateral elimination of the primary blood supply to one-half of the tongue. This study using rabbits was designed to ascertain the microvascular pattern in the collateral circulation of the tongue after unilateral ligation of the common cartoid artery. Results indicate that blood flow to the ligated side of the tongue was substantially reduced in the one hour experimental group. The six hour and twenty-four hour groups however, demonstrated only a slightly reduced flow. Animals in the one and two week groups displayed a microvascular perfusion pattern similar to that of the control animals who underwent no ligation.

These findings indicate that if ligation of a major vessel is necessary to control hemorrhage in traumatic injuries or surgical procedures in the tongue, collateral circulation is adequate to maintain the health of the tissue and a "normal" vascular pattern returns within one week after ligation.

Oral Manifestations of Tropical Diseases in Latin America

While oral diagnosis is strongly emphasized in most current American dental curricula, there is decidedly little emphasis placed on those conditions which are uncommon in the U.S., but which are of a much greater significance in other areas of the world, e.g. tropical diseases.

The experience of the U.S. Armed Forces with tuberculosis, bacterial pneumonia, veneral diseases, malaria, and intestinal infections during past wars has led to the development not only of wartime control of these and many other diseases, but also of peacetime prevention and decreased incidence in endemic areas. Still, during American involvement in Vietnam, more than twice as many man-days were lost due to disease than from war injuries. Knowledge or awareness of the association of oral manifestations with tropical diseases has not been highly documented. Recognition of signs or symptoms which may be present during dental examination would certainly enhance overall patient management.

Although the peacetime presence of American forces has existed in Latin America since before World War II, a sudden combat condition could necessitate the deployment of augmentative troops to the area. In such situations, diseases which may be relatively insignificant and well under control during peacetime may suddenly become of strategic concern during combat.

Examination of available past and current literature has enabled the development of a list of twenty-one diseases for inclusion in this group.

Selection of disease acties to be included were based on two criteria:

a) Significant incidence of the disease in the Latin American countries,
and b) The occurrence of oral manifestations which may be important to the
dentist not only from a treatment standpoint, but also because of diagnostic
relevance for these diseases. Material concerning the life cycles of
causative organisms (where appropriate), clinical course, oral manifestations, diagnosis, treatment, and military significance (where
relevant) has been completed for fifteen of the diseases to be researched.

Rapid Instrument Free Local Anesthesia of the Dental Pulp to Facilitate Treatment of Combat Injuries or Emergencies

Studies have continued on instrument free local anesthesia of the dental pulp. A ¹⁴C labeled DMSO-lidocaine combination was used on 22 freshly extracted human teeth. The teeth were thoroughly washed in saline and dried. The roots were removed and each tooth was placed, occlusal surface down, in a solution containing 70% dimethyl sulfoxide (DMSO) and 30% distilled water plus tagged carbonyl ¹⁴C lidocaine hydrochloride. The teeth were not emersed beyond the dentinoenamel junction. Distilled water was added to exposed pulp chambers at the time the teeth were emersed in the DMSO/H₂O/lidocaine solution. Aliquots were removed at selected time intervals for ¹⁴C counting by scintillation spectrometry. The data indicated ¹⁴C lidocaine did not penetrate the intact tooth within 60 minutes. In 4 teeth in which the enamel was not completely intact lidocaine penetrated the tooth within 5-10 minutes. This suggests that DMSO can assist in the transport of lidocaine into the pulp of teeth undergoing cavity preparation. Additional tests in which enamel will be partially removed from intact teeth and

subjected to ¹⁴C lidocaine solutions alone and in combination with DMSO will be done to determine if penetration of the anesthetic is quantitatively predictable and directly related to DMSO concentrations. Such a result would definitely support the possibility of instrument free local anesthesia of the dental pulp.

Attachment Levels on the Distal of Mandibular Second Molars Following Removal of Impacted Third Molars

Periodontal pocket formation on the distal of mandibular second molars following removal of partially erupted or impacted third molars is a common problem arising in military age patients. Although there is abundant evidence that periodontal disease occurs on mandibular second molars following removal of third molars in various positions and stages of development, literature is scarce in regard to surgical techniques which might prevent or minimize loss of periodontal attachment.

A great number of military personnel are at the age of partial third molar eruption or at the ideal age for impacted third molar removal. It would be significant to the military if a procedure could be developed that would prevent loss of functional attachment on the distal of second molars following removal of adjacent third molars. As a result, patients would experience fewer periodontal problems and less time away from duty.

The purpose of this study is to evaluate the periodontal attachment on the distal of second molars following removal of impacted and partially erupted third molars. The study will also attempt to determine if routine curettage of the socket associated with the third molar and the distal of

adjacent second molar will result in any change in periodontal attachment on the distal of mandibular second molars.

On the basis of an evaluation of one fourth of the data collected, the following conclusions were drawn:

- 1. Second molars lost attachment distally following removal of adjacent partially-erupted or tissue-covered third molars despite root planning of the second molars.
- 2. There was no statistical difference between the attachment levels on the distal of those second molars root planned to those that were not root planned.
- 3. Sulcular depths decreased in non-root planned second molars and increased in root planned second molars following removal of the partial or tissue-impacted third molars.
- 4. Although these conclusions are the result of only three-months data and calculations are presently being made on one year's data, it appears routine root planning of second molars following removal of adjacent partial or tissue-impacted third molars may be harmful rather than beneficial.

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12. KEYWORDS (Frecode BACH with Security Classification Code) (U) Subungal Bacteria; (U) Iodine Neutralizers (U) Free Fatty Acids; (U) Surgical Scrub; (U) Hydroscrub; (U) Radioactivity Decontami-23. TECHNICAL OBJECTIVE. 24. APPROACH, 25. PROGRESS (Furnish Individual paragraphs identified by number. Procedo test of each with Security CloselReation Code.) nation

23. (U) To determine the efficacy and application of pressurized water lavage to the treatment and prevention of disease in traumatic wounds, oral maxillofacial surgery and military dentistry.

- 24. (U) Specially designed instruments which yield water jets at different pressures and in both pulsating and nonpulsating streams are being used to study the effect of this modality on wound healing, dental plaque, and bacterial populations. The target tissue pressures with various water jet devices will be examined. The applicability to presurgical hand cleansing is being investigated.
- 25. (U) (77 10-78 10) Continued study of our hydroscrub system has shown that several reagents (Sodium sulfite, sodium thiosulfate and D/E neutralizing medium) used originally as part of the methodology for studying iodine agents added to hydroscrub, have themselves significant bacteria inhibiting effects. This line of investigation is continuing. Subungal decontamination remains a problem for hydroscrub. The significance of this and possible solutions are being studied. Correlation of skin free fatty acids with bacterial contamination continue to be unpromising. Decontamination of fine particulate matter from the hands using hydroscrub gave good results and the implication of this finding in whole-body lavage for removal of radioactive debris is being pursued.

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Role of Pressurized Water Lavage in the Practice of Military Dentistry.

Comparison of Iodine Inactivating Agents in Surgical Scrub Testing

We have previously reported that the commonly used inactivators of iodine, sodium thiosulfate, sodium sulfite, and D/E neutralizing medium, may have an inhibitory effect on bacterial growth. These iodine neutralizing agents incorporated into the culture media for the purpose of evaluating the effectiveness of iodine-containing surgical scrub preparations may produce false negative results because of their antibacterial effects and lead to misleading conclusions.

As we indicated in the last report, six reference microorganisms and ten bacterial strains isolated from the fingertips of two subjects were each affected to a different degree by the agents tested. Since no definite conclusion could be drawn at that time, we pursued our experiments further.

Additional microbial strains from fingertips were isolated and identified to be <u>Staphylococcus</u> epidermis, <u>Staphylococcus</u> aureus, <u>Bacillus</u> cereus, <u>Candida</u> stellatoidea, <u>Corynebacterium</u> xerosis, and three other gram positive rods to be further identified.

These organisms were tested for their susceptibility to the possible antimicrobial properties of the different concentrations of the three neutralizing agents used. The results indicated again that the growth of the majority of the microbial strains tested was inhibited most by sodium thiosulfate. Sodium sulfite and D/E neutralizing medium appeared to be generally less inhibitory, although sodium sulfite inhibited

almost completely the growth of <u>Candida stellatoidea</u>. It should also be pointed out that gram positive rods (to be identified) were completely inhibited even by very low concentrations of sodium thiosulfate, sodium sulfite, and D/E neutralizing medium. The implications of such findings during testing of surgical scrub preparations are obvious. Isolation of additional skin microbial strains and tests of their possible growth inhibition by neutralizing agents is in progress.

Effect of Surgical Preparation of Hands on Bacterial Concentrations Underneath the Fingernails

We have shown (Ann. Re. Prog. Rep. 1977) that bacterial concentrations underneath the fingernails remained very high following presurgical preparation of hands by two methods, 5 minute surgical scrub, and 90 second Hydroscrub developed by the U.S. Army Institute of Dental Research.

Since the majority of the subjects tested in the above study were laboratory personnel without previous experience in the routine surgical scrub techniques, obtained, the resulting bacterial counts following routine scrub could be attributed in part to the poor scrub technique, and it was considered appropriate to extend the testing to the additional group of participants. This group consisted of nine staff dentists who did have previous instruction and experience in proper surgical scrub procedures. They used both methods of presurgical preparation of hands on three occasions. The bacteriological cultures were obtained prior to and immediately after hand degerming.

It has been shown that the means for prescrub counts of 13.0×10^3 for the 10 minute routine scrub group and 20.7×10^3 for the Hydroscrub group were decreased after surgical scrub to 3.0×10^3 (76.9%) and 7.8×10^3 (63.3%), respectively.

Thus we have obtained convincing evidence on the basis of tests of 292 subungual areas of 20 subjects that surgical scrub by either of the methods tested does not reduce the microbial population under the fingernails of most individuals to "acceptable" levels.

It is obvious that the microbial counts in subungual areas remained very high, particularly in some individuals. Furthermore, on the basis of results of this study it is concluded that: (1) Degerming of the areas under the fingernails by present methods is not satisfactory; (2) Evaluation of the efficacy of various antiseptic agents and scrub techniques should include determination of the microbial counts in the subungual areas in addition to the assays of microbial population on the skin of hands; (3) The possible implication of the subungual microorganisms in the development of postsurgical infection should be investigated; and (4) A modification of the methods currently used, or possibly a new approach to the effective reduction of microbial population under the fingernails is necessary.

Rapid HPLC Method for the Determination of Fatty Acids on the $\operatorname{\mathsf{Hands}}$

It has been shown that certain fatty acids inhibit bacterial growth
This project was initiated to determine if people with low bacterial
concentrations on their hands had unique fatty acids present on the skin

of their fingers. Our results showed poor correlation between free fatty acids (FFA) on the hands and bacterial counts. Analysis of the individual free fatty acids showed positive correlation between all of the fatty acids analyzed and bacterial counts with the exception of one acid tentatively identified as either β -hydroxyl palmitic acid or 11-methyl tridecanoic acid. This acid had a negative correlation of 0.525 but it was not significant. Analysis of the post-surgical scrub chromatograms suggested that its origin is bacterial in nature. Further studies are planned to determine the antibacterial properties of this acid both "in vivo" and "in vitro".

The other fatty acids studied showed positive correlations ranging from 0.3 to 0.5 although none were significant at the 0.95 confidence level. This would indicate that although some of these acids had been identified to have antibacterial properties "in vitro", they do not demonstrate any antibacterial properties "in vivo" on the skin. Further studies are planned to confirm these findings.

Decontamination of Fine Particulater Matter From the Hands Using Hydroscrub

Potential contamination of scldiers with radioactive material during combat may create a serious problem that has to be dealt with effectively and as soon as possible following contamination.

Our previous experiments have shown that the Hand and Arm Washer, developed by USAIDR, effectively reduced bacterial counts on the hands of operating room personnel, and that this new method of hand degerming was superior to the conventional 10 min. brush scrub. Therefore, we investigated the feasibility of using our device for the purpose of

reducing contamination of the hands by adherent particulate matter which simulates dust as a potential carrier of radioactive material. Six subjects participated in this preliminary study. One gram of zinc oxide (ZnO) powder was placed on the hands and rubbed in.

The elimination of ZnO from hands was then attempted using pressure lavage (Hydroscrub device) at 120 psi for 1 min with (1) water, (2) aqueous dilution (250X) of povidone iodine, and (3) aqueous solution of Sparkle a commercial detergent (10 g per 103 L water). For comparison, hands were also rubbed with Rad-Con preparation (Radioactive Decontaminant Foam) for 1 min and rinsed with water thereafter. After decontamination the hands were dried with a cloth towel and samples of ZnO remaining on the fingertips were obtained. The samples were analyzed by energy dispersive x-ray analysis in the scanning electron microscope (electron probe) and atomic absorption spectrophotometry. The results have shown that both water and povidone iodine solution with pressure lavage did not remove ZnO from hands as effectively as Rad-Con. However, pressure lavage with Sparklene detergent solution may be as effective as Rad-Con application and is much faster and more easily adapted to whole body decontamination.

The results seem to warrant further decontamination tests which would utilize the high pressure water jet principle in a decontamination chamber to be constructed for that purpose.

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- 24. (U) The fate, metabolism, osteogenic potential and tissue compatibility of ceramic and copolymer materials will be studied alone and in combination. The application of these and other materials to avulsive type wounds in both animals and humans will be pursued.
- 25. (U) (77 10-78 10) An evaluation of titanium and vitallium mesh implants in function with dental prostheses in baboons indicate that they are well tolerated and may serve as substitutes for teeth in humans. X-ray microprobe and SEM analysis showed the implants entirely infiltrated with new bone. An evaluation of a biodegradable PLA-PGA mesh to support a particulate graft in a segmental mandibular defect has not given good results. The study will be repeated with 100% PLA. A study comparing biodegradable ceramic blocks with autogenous bone grafts as alveolar ridge augmenting agents is in progress. Human studies on the use of biodegradable ceramic to fill periodontal defects continue to be promising.

New and Improved Techniques for Grafts and Bone Regeneration in Traumatic Wounds

Titanium Implants in Baboons

SEM and energy-dispersive x-ray analysis was performed on contractorsupplied specimens of metal implants. Twelve implants of titanium and
vitallium metal, implanted in monkey jaws and in function with dental
prostheses, were sectioned, radiographed, and studied for bone growth
within the metal framework. Tetracycline fluorescent microscopy and
alizarin red vital staining showed new bone formation and remodeling
within the implant. X-ray microprobe and SEM study showed the implant
entirely infiltrated with new bone, especially at the base of the
implant where stress would have been most concentrated. Results indicate
that titanium and vitallium implant mesh is well tolerated and may serve
as a substitute for teeth in humans. Further study is indicated on this
technique and it's utilization in the military practice of dentistry.

Evaluation of a Biodegradable Mesh for Bone Grafts in the Dog Mandible

This study was designed to evaluate the feasibility of using a biodegradable mesh to support a particulate graft to the mandible for restoration of segmental defects seen in avulsive wounds. The initial results showed four (4) unions and two (2) non-unions in the mandible of the dog. Six (6) additional dogs were prepared for operation because of distortion seen in the biodegradable tray and screws used to stabilize the grafts.

We have been unable to obtain the necessary biodegradable material (100% PLA) to complete this study. However, dies for the fabrication of biodegradable trays have been cast from low-fusing base metal alloy and will be used upon receipt of the PLA.

Comparison of Biodegradable Ceramic Block and Autogenous Bone Grafts as Alveolar Ridge Augmentation Agents

This study was undertaken to compare the amount of ridge augmentation obtained by interposing 7mm blocks of tricalcium phosphate and 7mm blocks of bone between alveolar and basal bone in the dog's mandible.

Following an initial study on 15 dogs which provided us with conflicting results, we elected to repeat this study with change in flap design and mode of fixation of the elevated alveolar bone segment. A tongue flap in lieu of a semilunar flap was used to expose the operative site and nitinol springs replaced intraosseous wire for fixation. All but 3 animals have been sacrificed with marked improvement in clinical results. Histopathological evaluation will follow sacrifice of the remaining animals in November.

Utilization of Biodegradable Ceramics in Human Periodontal Defects

Work continues on the placement of biodegradable ceramic in human periodontal defects. To date ceramic implants have been placed in 46 osseous defects. Eight additional patients and 14 different sites are included in the present report. According to radiographic evidence there

appears to be approximately 3 mm of osseous regeneration in one, two and three wall defects. There is attachment present in one site where crestal bone was missing. However we will not be able to determine whether new crestal bone is actually present until a reentry procedure is performed. The first reentry procedure under the eighteen month reentry protocol is scheduled for October of 1978.

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- (U) Endodontic Procedures (U) X-ray Image Intensification
- 23. (U) Army endodontic procedures in the military total 108,000 per year and are 25% of dental energency procedures. Tooth reimplantation with endodontic therapy is involved in most serious facial injuries and involved typically 3 to 5 patient visits. The military can gain at least 50% reduction in patient and specialist man-hours spent in endodontic therapy with the development of more rapid and reliable treatment materials and techniques.
- 24. (U) Two areas to be investigated under this project are: (1) analysis of endodontic materials including those in use and newly developed; (2) techniques used in endodontic therapy with emphasis on the development of the most rapid and accurate method within the military type practice.
- 25. (U) (77 10-78 10) Evidence has been obtained that the routine use of hypochlorite in endodontic procedures may be contraindicated. Significant levels of metallic debris were found in teeth prepared by routine endodontic procedures. This could account for endodontic failures. An in-vitro study suggests that an ultrasonic probe may assist in root canal debridement. Data were obtained which indicate that citric acid cavity cleaners should be used more cautiously. A study of anesthetic cartridges sent from the field because of lack of potency revealed high levels of metal contamination. Evaluation of an X-ray image intensification apparatus designed at USAIDR indicates it may have potential for field use. A simulated study of root canal filling techniques in rats suggests that some accepted presautions are unnecessary. Tests designed to provide better guidelines for cutting efficiency of burs and stones are in progress.

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Development of Endodontic Procedures for Military Dentistry
Low Viscosity Embedding Resin Casts of Human Dentin
Tubules - An SEM Study

Ten partially or completely impacted third molars from a young adult military population were extracted as a part of regular treatment. coronal and apical regions of the pulp cavity were opened, and the teeth immersed in a 5% sodium hypochlorite solution in a sonication bath for 15 minutes. Subsequently, the teeth were washed in distilled water and infiltrated with a low viscosity TEM embedding resin at atmospheric pressure, heat cured, sectioned, decalcified, retreated in hypochlorite for 5 minutes without sonication, washed, mounted on SEM specimen stubs, coated with metal and examined in the SEM. Results indicated a complete penetration of resin to the D/E junction producing a striking view of the soft tissue spaces within the calcified dentin. Small linear spacial connections occurred between tubule systems. This technique study serves as a complementary method for the SEM supplementing anatomic observations from surface and fracture analyses. This study suggests that hypochlorite use may be a negative factor in the stability of endodontic preparations and as such have a negative effect on combat readiness.

Metal Deposits from Endodontic Instruments - An SEM and Microprobe Study

The root canal of 12 extracted human anterior teeth were enlarged with hand instruments and irrigated according to routine clinical procedures. The teeth were subsequently fractured, dehydrated, mounted on

specimen stubs, carbon-coated, and examined in an SEM for the entire surface of the dentin from apex to the area of the CEJ. Simultaneous energy-dispersive x-ray spectra were obtained from the same region. In 9 of 12 (75%) teeth, metal fragments emitting x-ray spectra identical to that of the hand instrument, were observed at the junction of the middle and apical thirds of the root. X-ray maps were superimposed on SEM micrographs. Particles ranged in size from 12 to 60 microns and were not distinguishable on SEM from dentin debris. This metal debris, located in the apical region of the canal, could be extruded into periapical tissue, serving as a potential cause of periapical inflammation and endodontic failures. Recognition of this potential would increase the success rate of Army Endodontic procedures.

Efficiency of Ultrasonic Debridement - A kadioisotope Study Using Simulated Root Canals

Evaluation of debridement techniques in endodontia involve predominantly subjective measures. Commercially available epon blocks containing simulated root canals have recently been developed for teaching these techniques. This study utilized these simulated canals, loaded with 1-125 and gelatin, as an objective standard for evaluating efficiency of hand instrumentation and hand instrumentation plus ultrasonication in removing debris from the pulp canal. Hand instrumentation technique removed 64% of the radiolabeled material from the canal utilizing either Na¹²⁵l or ¹²⁵l-labeled albumin in the gelatin mixture. Additional debridement with an ultrasonic probe produced a 78% (with Na¹²⁵l) and 88% (with ¹²⁵l-albumin) reduction in canal content.

The 22% to 40% increase in efficiency with sonication suggested that clinical evaluation of this technique should be initiated within the Army Dental Care Delivery System.

Effect of Citric Acid and Phosphoric Acid Etchant Solutions on Dentin Cavity Surfaces

Cavity walls and floors were examined in the SEM. The same areas were re-examined after creatment with one or both of the above cavity surface cleaning agents commonly used to prepare dental surfaces.

Before treatment, the cavity surfaces were covered with a "smeared" dentin layer, covering the openings of the dentinal tubules. Phosphoric acid did not affect this layer as seen with the SEM. Citric acid, however, dissolved the smeared layer and opened up the dentin tubules.

Clinically, this may indicate further penetration of the citric acid deeper into the dental pulp. Citric acid cavity cleansers should be used with caution if dentin is directly exposed to the acid. This information will be disseminated to the field.

SEM and X-Ray Analysis of Dental Anesthetic Cartridges

Anesthetic cartridges received from dental clinics Army-wide because of surface contamination and liquid particulate content were studied. Surface material showed aluminum chloride particles covering the needle entrance area of the rubber seal. Liquid was filtered, and the filters examined in the Philips 301 STEM. Initial results show large amounts of lead particles; also present was Barium, Sulfur, Titanium, Silicon,

Chlorine, and Calcium. This early study helps explain the failure of local anesthesia reported by 14 dental commanders.

Evaluation of X-Ray Image Intensification Apparatus

An x-ray image intensification apparatus was designed, utilizing an Army Night-Vision Laboratory image intensifier and a scintillator sheet from NASA to test the usefulness of x-ray radiography in dental applications. Endodontic files in place in dog molars, molar root anatomy and mandibular jaw fractures were radiographed with this apparatus and with clinical x-ray machines. Using Kodak Tri X film, exposure times of 1 - 5 seconds were required with the image intensifier. Radiographic detail was adequate, but inferior to present clinical equipment. However, the ability to view radiographic detail instantaneously with the intensifier, its portability and simplicity of design does show a great potential for military application in field use.

SEM-LM Analysis of the Intraosseous Response of ZOE-Filled Polyethylene Tube Implants

A primary factor in endodontic therapy is a knowledge of the reaction of the apical tissues to root canal obliterating substances. Since these materials remain permanently embedded, it is obvious that the methods inciting the least reaction are to be preferred. Previous studies have evaluated the connective tissue response to a multitude of canal sealers. However, studies evaluating the osseous tissue response are minimal. Sixty

250-300 gram Walter Reed strain rats were used to study the tissue inflammatory response to polyethylene tubes (PT) filled flush at both ends with fresh ZOE. The tibias of each animal were implanted with either a hollow PT control or one filled with ZOE. The animals were sacrificed in six groups of 10 each at 4, 7, 30, 90, 180, and 365 days following PT insertion. Observations by light and scanning electron microscopy revealed: 1. Approximate parallel osseous development occurred peripherally at the tube-tissue interface in both hollow and ZOE-filled implants. 2. Freshly prepared ZOE and the PT are non-irritating and are well tolerated by rat intraosseous tissue. 3. A canal filled flush at the apex with ZOE produces minimal or no inflammation. 4. No halo of irritation was noted, thus failing to confirm the hypothesis of a "hollow tube effect." A chi-square statistical analysis revealed a nonsignificant difference in the degree of inflammation between the two groups. A better knowledge of the factors critical to stable root canal preparations will lead to significant savings of costs expended in the placement of fixed and removable prostheses following tooth loss.

SEM Evaluation of Tungsten-Carbide Burs and Diamond Stones

Approximately 400,000 excavating burs and diamond stones are purchased annually by the U.S. Army Dental Corps, or approximately 180 burs per active duty dentist. No definitive guidelines presently exist as to when the cutting efficiency of dental rotary instruments is sufficiently reduced to dictate their replacement. SEM micrographs were

taken of the cutting portion of burs and stones as received from the manufacturer. These same instruments were then distributed to various dentists to be routinely used and collected following specified restorative procedures. The used instruments will then be re-photographed with SEM and evaluated. Establishment of guidelines for rotary instrument replacement should result in purchase reduction with subsequent savings of significant tax dollars.

Morphologic Analysis of the Mesiobuccal Root of the Maxillary First Molar Using SEM and Stereomicroscopy

Fifty-nine mesiobuccal roots of maxillary human molars were radiographed in two dimensions and evaluated under the stereomicroscope before and after horizontal sectioning 5mm from the apex. Fifty mesiobuccal roots were prepared for SEM analysis. The following parameters were recorded; root length, root configuration, canal number and configuration, apical foramina and resorptive defects. The findings revealed more variability than formerly reported in the literature. These results will be consolidated and published for general use.

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- (U) Biodegradable ceramic; (U) Segmental grafts; (U) Hollow Organ Grafts (U) Ureteral 23. TECHNICAL OBJECTIVE.* 24 APPROACH, 26 PROGRESS (Pumilia Individual paragraphs Identified by number. Proceed total of Security Classification Code.) Grafts 23. (U) To determine the best usage of biodegradable copolymer materials for the
- 23. (U) To determine the best usage of biodegradable copolymer materials for the treatment of both hard and soft tissue wounds. To develop premedicated biodegradable tissue fixation devices. To determine feasibility of a long-term, subcutaneous, slow release drug delivery system.
- 24. (U) The recent adaptation by USAIDR of the biodegradable copolymers to the surgical procedures such as tendon gliding devices, oral antral fistula closure and peripheral nerve repair will be expanded in animals and extended to man. Application to the Drug Review Board has been made for this usage.
- 25. (U) (77 10-78 10) Segmental ureteral grafts in dogs using biodegradable copolymers have been functional up to six months. Studies continue toward developing a more stable graft replacement with longer degradation times. The feasibility of esophageal grafts using biodegradable copolymers has been shown and studies continue with a new copolymer framework. The 2 year carcinogenesis study required for the biodegradable ceramic now in human use by USAIDR has not shown any deleterious effects or contraindications at the 19 month mark.

Biodegradable Materials for the Treatment of Fractures and Soft Tissue Wounds in the Military Situation

Segmental Ureteral Grafts

The left ureters in six mongrel dogs were utilized as a model for hollow organ regeneration. Two centimeter segments were removed and replaced with custom made soluble grafts constructed of several different copolymers designed to give degradation times of 1 to 6 weeks. Five grafts were well tolerated, slowly replaced, infiltrated with small areas of smooth muscle and became epithetialized. The sixth dog failed within the first week. Sacrifices over a six month period indicated a slow decrease in lumen size from 4 to 12 weeks. However the ureter was patent and functioning at 6 months. This model will be pursued in the quest for a more stable graft replacement in hollow organs with longer degradation times.

Esophageal Grafts

Feasibility studies have been completed on 5 rats and 2 dogs utilizing preformed grafts to stimulate neogenesis of new epithelial lined esophagi. These grafts were hand made and were composed of various soluble copolymers with degradation times between 7 days and 6 weeks. The rats regenerated esophagi in approximately 14 days and continued to do well up to 4 weeks. Both species were eventually blocked. However feasibility has been demonstrated by the replacement of the copolymers with fibrous connective tissue, the minimal ingrowth of smooth muscle and the regeneration of an epithelial lining. Further studies with a new copolymer framework are in progress.

Carcinogenesis Studies of Biodegradable Aramics

The FDA required 2 year carcinogenesis study of a complete state of amics in rats in continuing. The study will be completed by February 1979. There is no clinical evidence of any deleterious health effects to rate. Observations continue. The biodegradable ceramic is currently in use in our human use studies reported under accession number OH 6037

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